

L1 ANSWER 1 OF 1 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD  
 AN 89-138445 [19] WPIDS  
 C1C C89-061216  
 TI High yield microbial prodn. of 1,3-propane diol from glycerine -  
 using Klebsiella pneumoniae in media contg. cobalt salt and sugar.  
 DC A41 D16 E17  
 IN HILL, F F; TRANDINH, K  
 PA (CHEM) HUELS AG  
 CYC 1  
 PI DE 3734764 A 890503 (8919)\* 3 pp <--  
 ADT DE 3734764 A DE 87-3734764 871014  
 PRAI DE 87-3734764 871014  
 AB DE 3734764 A UPAB: 930923  
 Prodn. of 1,3-propanediol (I) comprises aerobic fermentation of  
 glycerine (II) with Klebsiella pneumonias DSM 4280 in presence of at  
 least one pentose or hexose and of divalent Co salts.  
 Fermentation is in presence of glucose and of 0.01-100, esp.  
 0.05-10 microM CoCl<sub>2</sub>, at 25-35 deg. C and pH 4-7, esp. 30-33 deg. C  
 and pH 4.5-6. Fermentation is in an aq. medium contg., initially,  
 5-15% (II) and 2-10% metabolisable carbohydrate, opt. with other  
 nutrients. After fermentation, the cells are removed and (I)  
 recovered from the liq. phase by distn. and fractional distn. Some  
 2,3-butanediol is formed as by product, the amt. depending on pH and  
 temp.  
 USE/ADVANTAGE - (I) is useful in prodn. of polyesters,  
 polyurethanes and special heterocyclic cpds. This method provides  
 high conversion and yields (e.g. 60-82g (I) per 100 g (II) and uses  
 renewable starting material.  
 0/0

L6 ANSWER 1 OF 2 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD  
 AN 91-303977 [42] WPIDS  
 DNC C91-131650  
 TI Anaerobic microbial conversion of substrate to metabolite - is in  
 airlift reactor with passage of inert gas.  
 DC D16 E17  
 IN CARDUCK, F J; DECKWER, W D; GUNZEL, B; KRETSCHMAN, J; YONSEL, S  
 PA (GBFB) GES BIOTECHNOL GBF; (HENK) HENKEL KGAA  
 CYC 15  
 PI DE 4010523 A 911010 (9142)\*  
 WO 9115590 A 911017 (9144) <--  
 RW: AT BE CH DE DK ES FR GB GR IT LU NL SE  
 W: JP US  
 ADT DE 4010523 A DE 90-4010523 900402  
 PRAI DE 90-4010523 900402  
 AB DE 4010523 A UPAB: 930928  
 In the microbial conversion of a substrate to a metabolite under  
 anaerobic conditions in a fermenter, (a) the fermenter is a  
 bubble-tube reactor with no mechanically moving inserts, and (b) a  
 gas free from O<sub>2</sub> is pressed into the lower region of the reactor  
 during the fermentation to convey the fermentation feed.  
 O<sub>2</sub>-free gases are the fermentation gases taken off at the head  
 of the reactor, and/or inert gases, e.g. N<sub>2</sub>, CO<sub>2</sub> or Ar. Rate of gas  
 feed is 0.001-0.2 (0.03-0.07) vvm, fed centrally (pref. axially) to  
 the bottom of the tower reactor through a pipe or a gasification  
 ring. Reactor pref. has a ratio of height:dia. of 5:20-10, and may  
 have static inserts promoting mixing, esp. recycling loops, which  
 are central or on the walls and act as sepn. wall. Prods. and/or  
 recycled culture medium is sprayed onto the foam, through a nozzle  
 in the upper part of the reactor, to control foam. Microorganism is  
 pref. Clostridium butyricum.  
 USE/ADVANTAGE - Useful for conversion of glycerol to propane  
 1,3-diol, using anaerobic micro-organisms. Foaming is low, (almost)  
 without use of an anti-foam.

L5 ANSWER 2 OF 2 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD  
 AN 90-100720 [14] WPIDS  
 CR 90-084142 [12]  
 DNC C90-044201  
 TI 1,3-Propane di ol prodn. by fermentation of aq. glycerine soln. -  
 with selected microorganism, then removal of biomass and distn. of  
 prod..  
 DC D16 E17  
 IN BIEBL, H; CARDUCK, F J; DECKWER, P; KRETSCHMAN, J; TAG, C; CARDUCK,  
 F; DECKWER, W; KRETSCHMANN, J  
 PA (GBFG) GES BIOTECH FORCHUNG; (GBFB) GES BIOTECH FORSCH GMBH; (HENK)  
 HENKEL KGAA; (GBFB) GBF GES BIOTECH FORSCHUNG GMBH  
 CYC 12  
 PI EP 361082 A 900404 (9014)\* DE 16 pp <--  
 R: AT BE CH DE ES FR GB IT LI NL  
 DK 8904231 A 900302 (9022)  
 DE 3924423 A 910131 (9106)  
 US 5254467 A 931019 (9343) 8 pp  
 ADT EP 361082 A EP 89-115555 890823; DE 3924423 A DE 89-3924423 890724;  
 US 5254467 A CIP of US 89-402209 890901, US 91-691648 910425  
 PRAI DE 88-3829618 880901; DE 89-3924423 890724  
 AB EP 361082 A UPAB: 931207  
 Process for conversion of glycerine into 1,3-propanediol by  
 microorganisms using a strain of microorganisms selected from  
 clostridium, Enterobacterium, Lactobacillus, Citrobacter, Aerobacter  
 and Klebsiella which is capable of converting glycerine into  
 1,3-propanediol in a space time yield of more than 0.5 g per hr. per  
 l in a 5 wt% glycerine soln. as sole carbon source under standard  
 fermentation conditions, comprises using the chosen microorganism  
 for conversion of a 5-20 wt%, (10-15 wt%) soln. of glycerine as  
 sole carbon source under anaerobic conditions while maintaining a  
 constant pH, and after extensive conversion of the glycerine, sepg.  
 obtd. biomass and working up the prod. mixt. by distn.  
 USE/ADVANTAGE - Used for technical scale use, esp. for prodn.  
 of 1,3-propanediol from glycerine waters obtd. from the industrial  
 processing of triglycerides, esp. glycerine solns. from the  
 saponification and/or transesterification of fats without  
 post-treatment of the glycerine-water phase.  
 Dwg.0/0

L5 ANSWER 1 OF 1 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD  
 AN 94-007553 [01] WPIDS  
 DNC C94-003076  
 TI Bacterial prod. for converting glycerol to 1,3-propane diol in high  
 yield - derived from new anaerobic strains of Enterobacter,  
 Corynebacterium or Citrobacter.  
 DC A41 B05 B07 D13 D16 D18 E17  
 IN BORIES, A; CLARET, C  
 PA (INRG) INST NAT RECH AGRONOMIQUE; (INRG) INRA INST NAT RECH  
 AGRONOMIQUE  
 CYC 17  
 PI WO 9325696 A1 931223 (9401)\* FR 34 pp <--  
 FR 2692281 A1 931217 (9403) 25 pp  
 EP 648273 A1 950419 (9520) FR  
 EP 648273 B1 960828 (9639) FR 14 pp  
 R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE  
 DE 69304332 E 961002 (9645)  
 ADT WO 9325696 A1 WO 93-FR568 930614; FR 2692281 A1 FR 92-7212 920615;  
 EP 648273 A1 EP 93-913124 930614, WO 93-FR568 930614; EP 648273 B1  
 EP 93-913124 930614, WO 93-FR568 930614; DE 69304332 E DE 93-604332  
 930614, EP 93-913124 930614, WO 93-FR568 930614  
 FDT EP 648273 A1 Based on WO 9325696; EP 648273 B1 Based on WO 9325696;

RAI FR 92-7212 920615  
B WO 9325696 A UPAB: 940217

Bacterial products (A) which can convert glycerol (I) to 1,3-propanediol (II) are prepd. by: (1) preculture of anaerobic populations, derived from anaerobic habitats, under anaerobic conditions on a buffered nutrient medium contg. (I) as sole carbon source; (2) isolating those precultures able to ferment (I); (3) enriching these precultures by discontinuous fermentation in an anaerobic reactor on (I)-based medium of controlled pH, and (4) isolating (A).

Also new are (A) themselves and the bacterial strains *Enterobacter agglomerans* CNCM I-1210 (most pref.); *Clostridium butyricum* I-1211 and *Citrobacter amalonaticus* I-1212.

USE/ADVANTAGE - (A) provide high yield conversion of (I) to (II) without significant by-product formation. (II) is used in synthesis of polyurethanes and polyesters; as an additive (esp. humectant) for foods and pharmaceuticals; in animal feeds; tobacco etc. (II) can now be produced from animal/plant waste materials, partic. by-products of alcohol distn.; avoiding the chemical synthesis from acrolein (which is toxic; derived from non-renewable resources and converted only with significant by-product formation).  
Dwg.0/5

9 ANSWER 1 OF 1 CAPLUS COPYRIGHT 1997 ACS

N 1994:126576 CAPLUS

N 120:126576

I \*\*\*Cloning\*\*\* and characterization of the propanediol dehydratase genes in \*\*\*Salmonella\*\*\* typhimurium

U \*\*\*Otto, Karin Elizabeth\*\*\*

S Texas Tech Univ., Lubbock, TX, USA

SO (1992) 77 pp. Avail.: Univ. Microfilms Int., Order No. DA9238983

From: Diss. Abstr. Int. B 1993, 53(8), 3921

OT Dissertation

LA English

AB Unavailable

11 ANSWER 3 OF 18 CAPLUS COPYRIGHT 1997 ACS

N 1992:446646 CAPLUS

N 117:46646

I Enhancement of 1,3-propanediol production by cofermentation in *Escherichia coli* expressing *Klebsiella pneumoniae* dha regulon genes

U \*\*\*Tong, I Teh\*\*\* ; Cameron, Douglas C.

S Dep. Chem. Eng., Univ. Wisconsin, Madison, WI, 53706-1691, USA

SO \*\*\*Appl\*\*\* . Biochem. Biotechnol. (1992), 34-35, 149-59

CODEN: ABIBDL; ISSN: 0273-2289

OT Journal

LA English

AB 1,3-Propanediol (I) is an intermediate in chem. and polymer synthesis. The genes of a biochem. pathway responsible for I prodn., the dha regulon of *K. pneumoniae*, have been previously expressed in *E. coli*. An anal. of the max. theor. yield of I from glycerol indicates that the yield can be improved by the cofermn. of sugars, provided that kinetic constraints are overcome. The yield of I from glycerol was improved from 0.46 mol/mol with glycerol alone to 0.63 mol/mol with glucose cofermn. and 0.55 mol/mol with xylose cofermn. The engineered *E. coli* also provides a model system for the study of metabolic pathway engineering.

12 ANSWER 3 OF 6 CAPLUS COPYRIGHT 1997 ACS

N 1990:419840 CAPLUS

N 113:19840

I Utilization of glycerol as a hydrogen acceptor by *Lactobacillus*

reuteri: purification of 1,3-propanediol:NAD<sup>+</sup> oxidoreductase  
 \*\*\*Talarico, Todd L.\*\*\* ; Axelsson, Lars T.; Novotny, James;  
 Fiuzat, Mitra; Dobrogosz, Walter J.  
 Dep. Microbiol., North Carolina State Univ., Raleigh, NC, 27695, USA  
 \*\*\*Appl\*\*\* . Environ. Microbiol. (1990), 56(4), 943-8  
 CODEN: AEMIDF; ISSN: 0099-2240  
 Journal  
 English  
 L. reuteri utilizes exogenously added glycerol as a H acceptor during carbohydrate fermns., resulting in higher growth rates and cell yields than those obtained during growth on carbohydrates alone. Glycerol is 1st converted to 3-hydroxypropionaldehyde by a coenzyme B12-dependent glycerol dehydratase and then reduced to 1,3-propanediol by an NAD-dependent oxidoreductase. The latter enzyme was purified and detd. to have a mol. wt. of 180,000; it is predicted to exist as a tetramer of identical 42,000-mol.-wt. subunits.

ANSWER 4 OF 6 CAPLUS COPYRIGHT 1997 ACS  
 1995:493624 CAPLUS  
 123:136863  
 Purification of 1,3-propanediol dehydrogenase from Citrobacter freundii and cloning; sequencing, and overexpression of the corresponding gene in Escherichia coli  
 \*\*\*Daniel, Rolf\*\*\* ; Boenigk, Rainer; Gottschalk, Gerhard  
 Institut fur Mikrobiologie, Georg-August-Universitat Gottingen, Gottingen, D-37077, Germany  
 J. \*\*\*Bacteriol\*\*\* . (1995), 177(8), 2151-6  
 CODEN: JOBAAY; ISSN: 0021-9193  
 Journal  
 English  
 1,3-Propanediol dehydrogenase (EC 1.1.1.202) was purified to homogeneity from Citrobacter freundii grown anaerobically on glycerol in continuous culture. The enzyme is an octamer of a polypeptide of 43,400 Da. When tested as a dehydrogenase, the enzyme was most active with substrates contg. 2 primary alc. groups sepd. by 1 or 2 carbon atoms. In the physiol. direction, 3-hydroxypropionaldehyde was the preferred substrate. The apparent Km values of the enzyme for 3-hydroxypropionaldehyde and NADH were 140 and 33 .mu.M, resp. The enzyme was inhibited by chelators of divalent cations but could be reactivated by the addn. of Fe<sup>2+</sup>. The dhaT gene, encoding the 1,3-propanediol dehydrogenase, was cloned, and its nucleotide sequence (1164 bp) was detd. The deduced dhaT gene product (387 amino acids, 41,324 Da) showed a high level of similarity to a novel family (type III) of alc. dehydrogenases. The dhaT gene was overexpressed in Escherichia coli 274-fold by using the T7 RNA polymerase/promoter system.

08/849404  
FILE 'REGISTRY' ENTERED AT 12:11:54 ON 09 DEC 1997  
L1 477 S HYDRATASE?

FILE 'MEDLINE' ENTERED AT 12:12:06 ON 09 DEC 1997  
L2 1 S L1

FILE 'REGISTRY' ENTERED AT 12:12:32 ON 09 DEC 1997  
L3 581 S DEHYDRATASE?

FILE 'MEDLINE' ENTERED AT 12:12:50 ON 09 DEC 1997  
L4 5 S L3

FILE 'REGISTRY' ENTERED AT 12:13:58 ON 09 DEC 1997  
L5 1 S GLYCEROL DEHYDRATASE  
L6 2 S DIOL DEHYDRATASE

FILE 'MEDLINE' ENTERED AT 12:14:36 ON 09 DEC 1997  
L7 0 S L5  
L8 0 S L6  
E DEHYDRATASES/CT  
E E4  
E DEHYDRATASE/CT  
E DEHYDRATASE/CN  
E HYDRO LYASES  
E HYDRO LYASES/CT  
L9 2938 S E9  
L10 77716 S CLONING, MOLECULAR/CT  
L11 125 S L9 AND L10  
L12 37111 S KLEBSIELLA OR LACTOBACILLUS OR ENTEROBACTER OR CITROBACTER OR PELOBACTER OR ILYOBACTER OR CLOSTRIDIUM  
L13 4 S L11 AND L12  
E GLYCEROL DEHYDRATASE  
E GLYCEROL DEHYDRATASE/CT  
E DIOL DEHYDRATASE  
E DIOL DEHYDRATASE/CT  
E DIOL DEHYDRATASE/CN  
E GLYCEROL DEHYDRATASE/CN  
L14 12 S E3  
L15 0 S L14 NOT L9  
L16 155 S L12 AND L9 NOT L13  
E KLEBSIELLA/CN  
E KLEBSIELLA/CT  
E L9  
E HYDRO LYASES/CT  
L17 291 S E22  
L18 7 S L17 AND L12  
L19 3 S L18 NOT L13

FILE 'SCISEARCH' ENTERED AT 12:32:16 ON 09 DEC 1997  
E SPRENGER G, 1989/RE  
E SPRENGER G A, 1989/RE  
L20 9 S E4

L4 ANSWER 1 OF 5 MEDLINE

T1 Site-directed mutagenesis of monofunctional chorismate mutase engineered from the E. coli P-protein.

L4 ANSWER 2 OF 5 MEDLINE

T1 Genetic aspects of aromatic amino acid biosynthesis in *Lactococcus lactis*.

L4 ANSWER 3 OF 5 MEDLINE

T1 The *pheA*/*tyrA*/*aroF* region from *Erwinia herbicola*: an emerging comparative basis for analysis of gene organization and regulation in enteric bacteria.

L4 ANSWER 4 OF 5 MEDLINE

T1 Loss of allosteric control but retention of the bifunctional catalytic competence of a fusion protein formed by excision of 260 base pairs from the 3' terminus of *gheA* from *Erwinia herbicola*.

L4 ANSWER 5 OF 5 MEDLINE

T1 Cloning, sequencing, and expression of the P-protein gene (*pheA*) of *Pseudomonas stutzeri* in *Escherichia coli*: implications for evolutionary relationships in phenylalanine biosynthesis.

L13 ANSWER 1 OF 4 MEDLINE

AN 96422012 MEDLINE

T1 Cloning, sequencing, and overexpression of the genes encoding coenzyme B12-dependent glycerol dehydratase of *Citrobacter freundii*.

AU Seyfried M; Daniel R; Gottschalk G

CS Institut für Mikrobiologie der Georg-August-Universität, Göttingen, Germany.

SO JOURNAL OF BACTERIOLOGY, (1996 Oct) 178 (19) 5793-6. Journal code: HH3. ISSN: 0021-9193. CY United States

DT Journal Article; (JOURNAL ARTICLE) LA English FS Priority Journals OS GENBANK-U09771 EM 9701 EW 19970104

AB The genes encoding coenzyme B12-dependent glycerol dehydratase of *Citrobacter freundii* were cloned and overexpressed in *Escherichia coli*. The B12-free enzyme was purified to homogeneity. It consists of three types of subunits whose N-terminal sequences are in accordance with those deduced from the open reading frames *dhaB*, *dhaC*, and *dhaE*, coding for subunits of 60,433 (alpha), 21,487 (beta), and 16,121 (gamma) Da, respectively. The enzyme complex has the composition  $\alpha_2\beta_2\gamma_2$ . Amino acid alignments with the subunits of the recently sequenced diol dehydratase of *Klebsiella oxytoca* (T. Tobimatsu, T. Hara, M. Sakaguchi, Y. Kishimoto, Y. Wada, M. Isoda, T. Sakai, and T. Toraya, J. Biol. Chem. 270:7142-7148, 1995) revealed identities between 51.8 and 70.9%.

CT Check Tags: Comparative Study

Bacterial Proteins: B1, biosynthesis

Bacterial Proteins: GE, genetics

Bacterial Proteins: IP, isolation & purification

Chromatography, Affinity

*Citrobacter freundii*: EN, enzymology

*Citrobacter freundii*: GE, genetics

Cloning, Molecular

Cobamides: ME, metabolism

*Escherichia coli*: GE, genetics

Genes, Bacterial

AN 89-138445 [19] WPIDS

DNC C89-061216

TI High yield microbial prodn. of 1,3-propane diol from glycerine -  
using Klebsiella pneumoniae in media contg. cobalt salt and sugar.

DC A41 D16 E17

IN HILL, F F; TRANDINH, K

PA (CHEM) HUELS AG

CYC 1

PI DE 3734764 A 890503 (8919)\* 3 pp <--

ADT DE 3734764 A DE 87-3734764 871014

PRAI DE 87-3734764 871014

AB DE 3734764 A UPAB: 930923

Prodn. of 1,3-propanediol (I) comprises aerobic fermentation of glycerine (II) with Klebsiella pneumonias DSM 4280 in presence of at least one pentose or hexose and of divalent Co salts.

Fermentation is in presence of glucose and of 0.01-100, esp. 0.05-10 microm CoCl<sub>2</sub>, at 25-35 deg. C and pH 4-7, esp. 30-33 deg. C and pH 4.5-6. Fermentation is in an aq. medium contg., initially, 5-15% (II) and 2-10% metabolisable carbohydrate, opt. with other nutrients. After fermentation, the cells are removed and (I) recovered from the liq. phase by distn. and fractional distn. Some 2,3-butanediol is formed as by product, the amt. depending on pH and temp.

USE/ADVANTAGE - (I) is useful in prodn. of polyesters, polyurethanes and special heterocyclic cpds. This method provides high conversion and yields (e.g. 60-82g (I) per 100 g (II) and uses renewable starting material.

0/0

\*\*\* Hydro-Lyases: BI, biosynthesis\*\*\*  
 \*\*\* Hydro-Lyases: GE, genetics\*\*\*  
 \*\*\* Hydro-Lyases: IP, isolation & purification\*\*\*  
 Molecular Sequence Data  
 Protein Conformation  
 Recombinant Proteins: BI, biosynthesis  
 Recombinant Proteins: IP, isolation & purification  
 Sequence Analysis, DNA  
 Sequence Homology, Amino Acid  
 Species Specificity  
 CN EC 4.2.1. (Hydro-Lyases); EC 4.2.1.30 (glycerol dehydratase); 0 (Bacterial Proteins); 0 (Cobamides); 0 (Recombinant Proteins)

## L13 ANSWER 2 OF 4 MEDLINE

AN 96394290 MEDLINE

TI Cloning, sequencing, and high level expression of the genes encoding adenosylcobalamin-dependent glycerol dehydratase of \*\*\*Klebsiella\*\*\* pneumoniae.

AU Tobimatsu T; Azuma M; Matsubara H; Takatori H; Niida T; Nishimoto K; Satoh H; Hayashi R; Toraya T

CS Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Tsushima-Naka, Okayama 700, Japan.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Sep 13) 271 (37) 22352-7. Journal code: HIV. ISSN: 0021-9258. CY United States DT Journal; Article; (JOURNAL ARTICLE)

LA English FS Priority Journals; Cancer Journals EM 9701 EW 19970104

AB The *gld* genes encoding adenosylcobalamin-dependent glycerol dehydratase of \*\*\*Klebsiella\*\*\* pneumoniae were cloned by cross-hybridization with a DNA fragment of \*\*\*Klebsiella\*\*\* oxytoca diol dehydratase genes. Since the *Escherichia coli* clones isolated did not show appreciable enzyme activity, plasmids for high level expression of cloned genes were constructed. The enzyme expressed in *E. coli* was indistinguishable from the wild-type glycerol dehydratase of *K. pneumoniae* by the criteria of polyacrylamide gelelectrophoretic, immunochemical, and catalytic properties. It was also shown that the recombinant functional enzyme consists of Mr 61,000, 22,000, and 16,000 subunits. Sequence analysis of the genes revealed four open reading frames separated by 2-12 bases. The sequential three open reading frames from the first to the third (*gldA*, *gldB*, and *gldC* genes) encoded polypeptides of 555, 194, and 141 amino acid residues with predicted molecular weights of 60,659(alpha), 21,355(beta), and 16,104(gamma), respectively. High level expression of these three genes in *E. coli* produced more than 14-fold higher level of fully active apoenzyme than that in *K. pneumoniae*. It was thus concluded that these are the genes encoding the subunits of glycerol dehydratase. The deduced amino acid sequences of the three subunits were 71, 58, and 54% identical with those of the alpha, beta, and gamma subunits of diol dehydratase, respectively, but failed to show any apparent homology with other proteins.

CT Check Tags: Support, Non-U.S. Gov't

Amino Acid Sequence  
 Base Sequence  
 \*\*\* Cloning, Molecular\*\*\*  
 DNA, Bacterial: CH, chemistry  
 Electrophoresis, Gel, Two-Dimensional  
 Escherichia coli  
 Gene Expression Regulation, Enzymologic  
 \*\*\* Hydro-Lyases: CH, chemistry\*\*\*  
 \*\*\* Hydro-Lyases: GE, genetics\*\*\*  
 \*\*\* Hydro-Lyases: ME, metabolism\*\*\*  
 \*\*\* Klebsiella pneumoniae: EN, enzymology\*\*\*  
 Molecular Sequence Data  
 Plasmids: ME, metabolism  
 Propanediol Dehydratase: CH, chemistry  
 Propanediol Dehydratase: GE, genetics  
 Propanediol Dehydratase: ME, metabolism  
 Restriction Mapping  
 Sequence Homology, Amino Acid  
 Sequence Homology, Nucleic Acid

CN EC 4.2.1. (Hydro-Lyases); EC 4.2.1.28 (Propanediol Dehydratase); EC 4.2.1.30 (glycerol dehydratase); 0 (DNA, Bacterial); 0 (Plasmids)

## L13 ANSWER 3 OF 4 MEDLINE

AN 93122543 MEDLINE

TI Growth temperature-dependent activity of glycerol dehydratase in *Escherichia coli* expressing the \*\*\*Citrobacter\*\*\* freundii *dha* regulon.

AU Daniel R; Gottschalk G

CS Institute für Mikrobiologie, Georg-August-Universität, Göttingen, FRG..

SO FEMS MICROBIOLOGY LETTERS, (1992 Dec 15) 79 (1-3) 281-5. Journal code: FML. ISSN: 0378-1097. CY Netherlands DT Journal; Article; (JOURNAL ARTICLE)

LA English FS Priority Journals EM 9304

AB Using the cosmid pWE15, a genomic library of \*\*\*Citrobacter\*\*\* freundii DNA in *Escherichia coli* ECL707 was prepared and screened for glycerol utilization. Six out of approximately 3000 clones were positive. One clone, harboring the recombinant cosmid pRD1, expressed glycerol dehydratase in high activity when grown at 28 degrees C but not at 37 degrees C. The growth temperature had little effect on the activity of the other enzymes encoded by the *dha* regulon. When the glycerol-containing medium was supplemented with cornitins, the recombinant *E. coli* strain produced 1,3-propanediol in high amounts at 28 degrees C.

CT Check Tags: Support, Non-U.S. Gov't

\*\*\* Citrobacter freundii: EN, enzymology\*\*\*  
 \*\*\* Citrobacter freundii: GE, genetics\*\*\*  
 \*\*\* Cloning, Molecular\*\*\*  
 \*Escherichia coli: EN, enzymology  
 Escherichia coli: GD, growth & development  
 Escherichia coli: GE, genetics  
 Genes, Bacterial  
 Genes, Regulator  
 Glycerin: ME, metabolism  
 \*\*\* Hydro-Lyases: GE, genetics\*\*\*  
 \*\*\* Hydro-Lyases: ME, metabolism\*\*\*  
 Propanediols: ME, metabolism  
 Temperature

RN 504-63-2 (1,3-propanediol); 56-81-5 (Glycerin)

CN EC 4.2.1. (Hydro-Lyases); EC 4.2.1.30 (glycerol dehydratase); 0 (Propanediols)

GEN *dha*

## L13 ANSWER 4 OF 4 MEDLINE

AN 92412068 MEDLINE

TI Cloning and properties of a cyanide hydratase gene from the phytopathogenic fungus *Gloeocercospora sorghi*.

AU Wang P; VanEtten H D

CS Department of Plant Pathology, University of Arizona, Tucson 85721..

SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1992 Sep 16) 187 (2) 1048-54. Journal code: 9YB. ISSN: 0006-291X. CY United States

DT Journal; Article; (JOURNAL ARTICLE) LA English FS Priority Journals; Cancer Journals

OS GENBANK-M99044; GENBANK-S41678; GENBANK-S41679; GENBANK-S41680; GENBANK-S41731; GENBANK-D10916; GENBANK-D10917; GENBANK-D10918; GENBANK-D10919; GENBANK-D10920 EM 9212

AB The *cht* gene encoding cyanide hydratase (CHT, EC 4.2.1.66), which detoxifies HCN and is thought to be important in fungal infection of cyanogenic plants, has been cloned from the phytopathogenic fungus *Gloeocercospora sorghi*. The gene was isolated by screening an expression library of *G. sorghi* using a CHT-specific antibody and using one of the positive cDNA clones as a probe in Southern hybridization to identify a 3.1 kb *Pst*I genomic fragment. This *Pst*I fragment expressed CHT activity when transformed into *Aspergillus nidulans*, a fungus that normally lacks CHT activity. Sequence analysis identified a single open reading frame of 1,107 base pairs which encodes a polypeptide of 40,904 daltons. The deduced amino acid sequence of CHT shares 36.5% identity to a nitrilase from the bacterium \*\*\*Klebsiella\*\*\* pneumoniae subsp. *ozaenae*.

CT Check Tags: Comparative Study

Amino Acid Sequence  
 Aminohydrolases: CH, chemistry  
 Aspergillus nidulans: GE, genetics  
 Base Sequence  
 Blotting, Southern  
 \*\*\* Cloning, Molecular\*\*\*  
 DNA: CH, chemistry  
 DNA: IP, isolation & purification  
 DNA Probes  
 \*\*\* Hydro-Lyases: CH, chemistry\*\*\*  
 \*\*\* Hydro-Lyases: GE, genetics\*\*\*  
 \*Hyphomycetes: EN, enzymology  
 Hyphomycetes: GE, genetics  
 \*\*\* Klebsiella pneumoniae: EN, enzymology\*\*\*

Molecular Sequence Data  
Nucleic Acid Hybridization  
Poly A: GE, genetics  
Potassium Cyanide: PD, pharmacology  
RNA: GE, genetics  
Sequence Homology, Nucleic Acid  
Transcription, Genetic  
Transformation, Genetic  
Translation, Genetic

RN 151-50-8 (Potassium Cyanide); 24937-83-5 (Poly A); 63231-63-0 (RNA); 9007-49-2 (DNA)  
CN EC 3.5.4. (Aminohydrolases); EC 3.5.5.1 (nitrilase); EC 4.2.1. (Hydro-Lyases); EC 4.2.1.66 (cyanide hydratase); 0 (DNA Probes); 0 (RNA, Messenger)

## L19 ANSWER 1 OF 3 MEDLINE

TI Analysis of acyl coenzyme A binding to the transcription factor FadR and identification of amino acid residues in the carboxyl terminus required for ligand binding.

## L19 ANSWER 2 OF 3 MEDLINE

TI The nucleotide sequence of genes involved in the leucine biosynthetic pathway of \*\*\*Clostridium\*\*\* pasteurianum.

## L19 ANSWER 3 OF 3 MEDLINE

TI Anaerobic growth of *Escherichia coli* on glycerol by importing genes of the *dha* regulon from \*\*\*Klebsiella\*\*\* pneumoniae.

## L19 ANSWER 3 OF 3 MEDLINE

AN 90155202 MEDLINE

TI Anaerobic growth of *Escherichia coli* on glycerol by importing genes of the *dha* regulon from \*\*\*Klebsiella\*\*\* pneumoniae.

AU Sprenger G A; Hammer B A; Johnson E A; Lin E C

CS Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

NC 5-RO1-GM11983 (NIGMS)

SO JOURNAL OF GENERAL MICROBIOLOGY, (1989 May) 135 ( Pt 5) 1255-62. Journal code: 187. ISSN: 0022-1287. CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE) LA English FS Priority Journals EM 9005

AB The *dha* regulon of \*\*\*Klebsiella\*\*\* pneumoniae specifying fermentative dissimilation of glycerol was mobilized by the broad-host-range plasmid RP4:mini Mu and introduced conjugatively into *Escherichia coli*. The recipient *E. coli* was enabled to grow anaerobically on glycerol without added hydrogen acceptors, although its cell yield was less than that of *K. pneumoniae*. The reduced cell yield was probably due to the lack of the coenzyme-B12-dependent glycerol dehydratase of the *dha* system. This enzyme initiates the first step in an auxiliary pathway for disposal of the extra reducing equivalents from glycerol. The lack of this enzyme would also account for the absence of 1,3-propanediol (a hallmark fermentation product of glycerol) in the spent culture medium. In a control experiment, a large quantity of this compound was detected in a similar culture medium following the growth of *K. pneumoniae*. The other three known enzymes of the *dha* system, glycerol dehydrogenase, dihydroxyacetone kinase and 1,3-propanediol oxidoreductase, however, were synthesized at levels comparable to those found in *K. pneumoniae*. Regulation of the *dha* system in *E. coli* appeared to follow the same pattern as in *K. pneumoniae*: the three acquired enzymes were induced by glycerol, catabolite repressed by glucose, and glycerol dehydrogenase was post-translationally inactivated during the shift from anaerobic to aerobic growth. The means by which the *E. coli* recipient can achieve redox balance without formation of 1,3-propanediol during anaerobic growth on glycerol remains to be discovered.

CT Check Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Alcohol Oxidoreductases: GE, genetics

Anaerobiosis

Bacterial Proteins: GE, genetics

Conjugation, Genetic

Energy Metabolism

\**Escherichia coli*: GD, growth & development

*Escherichia coli*: GE, genetics

*Escherichia coli*: ME, metabolism

Fermentation

\*Genes, Bacterial

Glycerin: ME, metabolism

\*\*\*Hydro-Lyases: GE, genetics\*\*\*

Hydro-Lyases: PH, physiology

\*\*\*Klebsiella pneumoniae: GE, genetics\*\*\*

Oxidation-Reduction

Phosphotransferases: GE, genetics

Plasmids

Sugar Alcohol Dehydrogenases: GE, genetics

RN 56-81-5 (Glycerin)

CN EC 1.1 (Alcohol Oxidoreductases); EC 1.1. (Sugar Alcohol Dehydrogenases); EC 1.1.1.6 (glycerol dehydrogenase); EC 1.1.1.77 (lactaldehyde reductase); EC 2.7 (Phosphotransferases); EC 2.7.1.29 (glycerone kinase); EC 4.2.1. (Hydro-Lyases); EC 4.2.1.30 (glycerol dehydratase); 0 (Bacterial Proteins); 0 (Plasmids)

## L20 ANSWER 1 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI Glycerol conversion to 1,3-propanediol by *Clostridium pasteurianum*: cloning and expression of the gene encoding 1,3-propanediol dehydrogenase

## L20 ANSWER 2 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI Structure and gene-polypeptide relationships of the region encoding glycerol diffusion facilitator (gfpF) and glycerol kinase (gfpK) of *Pseudomonas aeruginosa*

## L20 ANSWER 3 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THE OXIDATIVE BRANCH OF GLYCEROL UTILIZATION BY *CITROBACTER-FREUNDII*

## L20 ANSWER 4 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI CLONING AND NUCLEOTIDE-SEQUENCE OF THE GLPD GENE ENCODING SN-GLYCEROL-3-PHOSPHATE DEHYDROGENASE OF *PSEUDOMONAS-AERUGINOSA*

## L20 ANSWER 5 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI DIOL DEHYDRASE AND GLYCEROL DEHYDRASE, COENZYME B-12-DEPENDENT ISOZYMES

## L20 ANSWER 6 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI MAPPING AND CLONING OF GLDA, THE STRUCTURAL GENE OF THE *ESCHERICHIA-COLI* GLYCEROL DEHYDROGENASE

## L20 ANSWER 7 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI ANALYSIS OF THE *ESCHERICHIA-COLI* GENOME. 4. DNA-SEQUENCE OF THE REGION FROM 89.2 TO 92.8 MINUTES

## L20 ANSWER 8 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI GROWTH TEMPERATURE-DEPENDENT ACTIVITY OF GLYCEROL DEHYDRATASE IN *ESCHERICHIA-COLI* EXPRESSING THE *CITROBACTER-FREUNDII* *DHA* REGULON

## L20 ANSWER 9 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

TI 1,3-PROPANEDIOL PRODUCTION BY *ESCHERICHIA-COLI* EXPRESSING GENES FROM THE *KLEBSIELLA-PNEUMONIAE*-*DHA* REGULON

## L20 ANSWER 1 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

AN 97:718159 SCISEARCH

GA The Genuine Article (R) Number: XX393

TI Glycerol conversion to 1,3-propanediol by *Clostridium pasteurianum*: cloning and expression of the gene encoding 1,3-propanediol dehydrogenase

AU Luers F; Seyfried M; Daniel R; Gottschalk G (Reprint)

CS UNIV GOTTINGEN, INST MIKROBIOL, GRIEBACHSTR 8, D-37077 GOTTINGEN, GERMANY (Reprint); UNIV GOTTINGEN, INST MIKROBIOL, D-37077 GOTTINGEN, GERMANY

CYA GERMANY

SO FEMS MICROBIOLOGY LETTERS, (15 SEP 1997) Vol. 154, No. 2, pp. 337-345. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

ISSN: 0378-1097. DT Article; Journal FS LIFE LA English REC Reference Count: 34

AB When grown on glycerol as sole carbon and energy source, cell extracts of *Clostridium pasteurianum* exhibited activities of glycerol dehydrogenase, dihydroxyacetone kinase, glycerol dehydratase and 1,3-propanediol dehydrogenase. The genes encoding the latter two enzymes were cloned by colony hybridization using the *dhaT* gene of *Citrobacter freundii* as heterologous DNA probe and expressed in *Escherichia coli*. The native molecular mass of 1,3-propanediol dehydrogenase (*DhaT*) is 440 000 Da. The *dhaT* gene of *C. pasteurianum* was subcloned and its nucleotide sequence (1158 bp) was determined. The deduced gene product (41 776 Dal revealed high similarity to *DhaT* of *C. freundii* (80.5% identity; 89.8% similarity).

CC MICROBIOLOGY

ST Author Keywords: *Clostridium pasteurianum*; 1,3-propanediol dehydrogenase; 1,3-propanediol; glycerol fermentation; type III alcohol dehydrogenase; glycerol dehydratase

STP KeyWords Plus (R): *ESCHERICHIA-COLI*; ALCOHOL-DEHYDROGENASE; *CITROBACTER-FREUNDII*; MOLECULAR CHARACTERIZATION; *KLEBSIELLA-PNEUMONIAE*; *ZYMOMONAS-MOBILIS*; SEQUENCE-ANALYSIS; *DHA* REGULON; PROTEIN

OVEREXPRESSION

RF 95-0536 001; 11-BETA-HYDROXYSTEROID DEHYDROGENASE; FETAL ORIGINS OF CORONARY HEART-DISEASE; APPARENT MINERALOCORTICOID EXCESS SYNDROMES



95-3190 001; INCREASED ABUNDANCE OF SPECIFIC SKELETAL-MUSCLE KINASE-TYROSINE PHOSPHATASES; ALPHA-B-CRYSTALLIN EXPRESSION  
95-3375 001; THERMUS STRAINS; DNA RELATEDNESS; GENUS AEROMONAS; EMENDED DESCRIPTION OF CAMPYLOBACTER-HYDROLYTICUS; POLYPHASIC TAXONOMY  
95-5061 001; STRUCTURAL GENE; GLYC-DEPENDENT REGULATION OF BACILLUS-SUBTILIS GLUTAMATE SYNTHASE EXPRESSION; ARABIDOPSIS TYPE-1 PROTEIN PHOSPHATASE

RE

Referenced Author (RAU)	[Year] [RPY]	[VOL] [RVL]	[PG] [RPG]	[Referenced Work] [RWK]
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ABBADANDALOUSSI S	1996	142	1149	J MICROBIOL-UK
ANDERSSON L O	1972	120	119	J FEBS LETT
AUSUBEL F M	1987	1	1	J CURRENT PROTOCOLS MO
BAIROCH A	1991	119	2241	J NUCLEIC ACIDS RES
BOENIGK R	1991	1	1	J THESIS G AUGUST U GO
BRADFORD M M	1976	172	248	J ANAL BIOCHEM
CONWAY T	1987	169	2591	J BACTERIOL
CONWAY T	1989	171	3754	J BACTERIOL
DABROCK B	1992	158	1233	J APPL ENVIRON MICROB
DANIEL R	1992	100	281	J FEMS MICROBIOL LETT
DANIEL R	1995	177	2151	J BACTERIOL
DANIEL R	1995	177	4392	J BACTERIOL
DEVRIES G E	1992	174	5346	J BACTERIOL
FISCHER R J	1993	175	16659	J BACTERIOL
GOODLOVE P E	1989	185	209	J GENE
HEYNDRIKX M	1991	134	1637	J APPL MICROBIOL BIOT
HOMANN T	1990	133	1121	J APPL MICROBIOL BIOT
JOHNSON E A	1984	160	155	J BACTERIOL
KELL D B	1981	199	181	J BIOCHEM BIOPH RES CO
KESSLER D	1992	267	18073	J BIOL CHEM
MARMUR J	1961	13	208	J MOL BIOL
REID M F	1994	120	113	J CRIT REV MICROBIOL
RUCH F E	1974	119	150	J BACTERIOL
SANGER F	1977	174	15463	J NATL ACAD SCI USA
SEYFRIED M	1996	178	15793	J BACTERIOL
SOHLING B	1996	178	1871	J BACTERIOL
SPRENGER G A	1989	135	1255	J GEN MICROBIOL
STOJILJKOVIC I	1995	177	1357	J BACTERIOL
TORAYA T	1977	252	1963	J BIOL CHEM
TSE P	1988	110	1295	J AM CHEM SOC
WALTER K A	1992	174	17149	J BACTERIOL
WIERENGA R K	1985	124	1346	J BIOCHEMISTRY-US
WILLIAMSON V M	1987	209	1374	J MOL GEN GENET
YOUNGLESON J S	1988	178	1355	J GENE

L20 ANSWER 3 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

AN 95:524431 SCISEARCH

GA The Genuine Article (R) Number: RL828

TI BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THE OXIDATIVE BRANCH OF GLYCEROL UTILIZATION BY CITROBACTER-FREUNDII

AU DANIEL R; STUERTZ K; GOTTSCHALK G (Reprint)

CS UNIV GOTTINGEN, INST MIKROBIOL, GRISEBACHSTR 8, D-37077 GOTTINGEN, GERMANY (Reprint); UNIV GOTTINGEN, INST MIKROBIOL, D-37077 GOTTINGEN, GERMANY CYA GERMANY

SO JOURNAL OF BACTERIOLOGY, (AUG 1995) Vol. 177, No. 15, pp. 4392-4401. ISSN: 0021-9193. DT Article; Journal FS LIFE A ENGLISH REC Reference Count: 58

AB Glycerol dehydrogenase (EC 1.1.1.6) and dihydroxyacetone kinase (EC 2.7.1.29) were purified from *Citrobacter freundii*. The dehydrogenase is a hexamer of a polypeptide of 43,000 Da. The enzyme exhibited a rather broad substrate specificity, but glycerol was the preferred substrate in the physiological direction. The apparent  $K_m$ (s) of the enzyme for glycerol and NAD(+) were 1.27 mM and 57  $\mu$ M, respectively. The kinase is a dimer of a polypeptide of 57,000 Da. The enzyme was highly specific for the substrates dihydroxyacetone and ATP; the apparent  $K_m$ (s) were 30 and 70  $\mu$ M, respectively. The DNA region which contained the genes encoding glycerol dehydrogenase (dhaD) and dihydroxyacetone kinase (dhaK) was cloned and sequenced. Both genes were identified by N-terminal sequence comparison. The deduced dhaD gene product (365 amino acids) exhibited high degrees of homology to glycerol dehydrogenases from other organisms and less homology to type III alcohol dehydrogenases, whereas the dhaK gene product (552 amino acids) revealed no significant homology to any other protein in the databases. A large gene (dhaR) of 1,929 bp was found downstream from dhaD. The deduced gene product (641 amino acids) showed significant similarities to members of the sigma(54) bacterial enhancer-binding protein family.

CC MICROBIOLOGY

STP KeyWords Plus (R): ACTIVATED ALCOHOL-DEHYDROGENASE; METAL DISSOCIATION-CONSTANTS; ESCHERICHIA-COLI; KLEBSIELLA-PNEUMONIAE; ZYMOMONAS-MOBILIS; SACCHAROMYCES-CEREVISIAE; NUCLEOTIDE-SEQUENCE; D

REGULON; BACILLUS-STEAROTHERMOPHILUS; REGULATORY GENE

RF 93-4847 004; HETEROLOGOUS EXPRESSION; CHROMOSOMAL DNA; GENE ENCODING METHYLMALONYL-COENZYME-A MUTASE

93-7500 002; PROTEIN PHOSPHATASE-1; PHOTOTROPHIC BACTERIUM RHODOBACTER-CAPSULATUS E1F1; CALF UTERUS

93-3088 001; RAT MUSCLE; PROTEIN PHOSPHATASE-1; MAJOR GLUTATHIONE TRANSFERASE

93-6277 001; ESCHERICHIA-COLI MESSENGER-RNA PROMOTER SEQUENCES; TRANSCRIPTION INITIATION; EXPRESSION OF THE CELLULOMONAS-FLAVIGENA CELL-ASSOCIATED AMYLASE GENE

93-7923 001; SULFATE-REDUCING BACTERIUM; ANAEROBIC DEGRADATION; METHANE FORMATION

RE

Referenced Author (RAU)	[Year] [RPY]	[VOL] [RVL]	[PG] [RPG]	[Referenced Work] [RWK]
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ARNOLD W	1988	203	1715	J MOL BIOL
BAIROCH A	1991	119	2241	J NUCLEIC ACIDS RES
BLATTNER F R	1993	21	15408	J NUCLEIC ACIDS RES
BLUM H	1987	18	193	J ELECTROPHORESIS
BOENIGK R	1993	138	1453	J APPL MICROBIOL BIOT
BRADFORD M M	1976	172	248	J ANAL BIOCHEM
CONWAY T	1987	169	2591	J BACTERIOL
CONWAY T	1989	171	3754	J BACTERIOL
DANIEL R	1992	100	281	J FEMS MICROBIOL LETT
DANIEL R	1995	177	2151	J BACTERIOL
DEVEREUX J	1984	112	1387	J NUCLEIC ACIDS RES
DEVRIES G E	1992	174	5346	J BACTERIOL
DREWKE C	1988	1950	154	J BIOCHIM BIOPHYS ACTA
FISCHER R J	1993	175	16659	J BACTERIOL
FORAGE R G	1982	151	1591	J BACTERIOL
FRY D C	1986	183	1907	J NATL ACAD SCI USA
GOODLOVE P E	1989	185	209	J GENE
HAWLEY D K	1983	111	2237	J NUCLEIC ACIDS RES
HOMANN T	1990	133	1121	J APPL MICROBIOL BIOT
INOUE S	1988	166	1301	J GENE
JOHNSON E A	1984	160	155	J BACTERIOL
KESSLER D	1992	267	18073	J BIOL CHEM
KRUGER N	1992	174	14391	J BACTERIOL
KYHSEANDERSEN J	1984	110	203	J BIOCHEM BIOPH METH
LAEMMLI U K	1970	270	1680	J NATURE
MALLINDER P R	1992	110	19	J GENE
MARCK C	1988	116	11829	J NUCLEIC ACIDS RES
MARTIN R G	1961	236	11372	J BIOL CHEM
MORETT E	1993	175	16067	J BACTERIOL
PETTIGREW D W	1988	263	1135	J BIOL CHEM
PFENNIG N	1986	155	245	J ARCH MIKROBIOL
RAMAKRISHNAN G	1990	187	2369	J NATL ACAD SCI USA
REID M F	1994	120	113	J CRIT REV MICROBIOL
RUCH F E	1974	119	150	J BACTERIOL
RUCH F E	1980	141	1077	J BACTERIOL
SAMBROOK J	1989	1	1	J MOL CLONING LABORATO
SANGER F	1977	174	15463	J NATL ACAD SCI USA
SHINE J	1974	171	1342	J NATL ACAD SCI USA
SHINGLER V	1993	175	15596	J BACTERIOL
SIEGEL L M	1966	112	1346	J BIOCHIM BIOPHYS ACTA

SOHLING B | | | JUNPUB  
 SPENCER P |1989 |994 |270 |BIOCHIM BIOPHYS ACTA  
 SPRENGER G A |1989 |135 |1255 |J GEN MICROBIOL <-  
 SRIDHARA S |1969 |98 |87 |J BACTERIOL  
 SUTCLIFFE J G |1979 |43 |77 |COLD SPRING HARB SYM  
 TANG C T |1979 |140 |182 |J BACTERIOL  
 THORNER J W |1971 |246 |3885 |J BIOL CHEM  
 TONG I T |1991 |57 |3541 |APPL ENVIRON MICROB  
 TORAYA T |1977 |252 |963 |J BIOL CHEM  
 TSE P |1988 |110 |1295 |J AM CHEM SOC  
 TSE P |1989 |111 |8703 |J AM CHEM SOC  
 VIEIRA J |1982 |19 |259 |GENE  
 WALTER K A |1992 |174 |7149 |J BACTERIOL  
 WANG A Y |1992 |31 |11020 |BIOCHEMISTRY-US  
 WILLIAMSON V M |1987 |209 |374 |MOL GEN GENET  
 YAMADA H |1982 |46 |2333 |AGR BIOL CHEM TOKYO  
 YANISCHPERRON C |1985 |33 |103 |GENE  
 YOUNGLESON J S |1988 |78 |355 |GENE

L20 ANSWER 5 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

AN 94:207853 SCISEARCH

GA The Genuine Article (R) Number: BZ91T

TI DIOL DEHYDRASE AND GLYCEROL DEHYDRASE, COENZYME B-12-DEPENDENT ISOZYMES

AU TORAYA T (Reprint)

CS OKAYAMA UNIV, FAC ENGN, DEPT BIOTECHNOL, 3-1-1 TSUSHIMA NAKA, OKAYAMA 700, JAPAN (Reprint)

CYA JAPAN

SO METAL IONS IN BIOLOGICAL SYSTEMS, (1994) Vol. 30, pp. 217-254. ISSN: 0161-5149. DT General Review, Journal LA ENGLISH REC Reference Count: 110

CC CHEMISTRY, INORGANIC & NUCLEAR; BIOLOGY, MISCELLANEOUS; BIOCHEMISTRY & MOLECULAR BIOLOGY; BIOPHYSICS

STP KeyWords Plus (R): BOND-DISSOCIATION ENERGY; CARBON-COBALT BOND; CO-C BOND; KLEBSIELLA-PNEUMONIAE; CHEMICAL MODIFICATION; ESCHERICHIA-COLI; DHA REGULON; D-RIBOSE; ADENOSYLCOBALAMIN; ENZYME

RE

Referenced Author (RAU)	Year (RPA)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)
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ABELES R H	1976	9	114	ACCOUNTS CHEM RES
ABELES R H	1964	112	695	JANN NY ACAD SCI
ABELES R H	1960	41	531	BIOCHIM BIOPHYS ACTA
ABELES R H	1971	5	481	ENZYMES
ABELES R H	1961	236	2347	J BIOL CHEM
ABELES R H	1966	241	1245	J BIOL CHEM
ABELES R H	1966	9	686	METHOD ENZYMOL
ABELES R H	1979	1	373	VITAMIN B12
ANTON D L	1980	102	2215	J AM CHEM SOC
ANTON D L	1980	255	4507	J BIOL CHEM
BABIOR B M	1988	1	21	BIOFACTORS
BABIOR B M	1974	249	1689	J BIOL CHEM
BACHOVCHIN W W	1977	16	1082	BIOCHEMISTRY-US
BACHOVCHIN W W	1978	17	2218	BIOCHEMISTRY-US
BONT J A M	1982	174	465	BIOCHIM BIOPHYS ACTA
BROWNSTEIN A M	1961	236	1199	J BIOL CHEM
COCKLE S A	1972	94	275	J AM CHEM SOC
DANIEL R	1992	100	281	FEMS MICROBIOL LETT
EAGAR R G	1975	14	5523	BIOCHEMISTRY-US
EBERHARD G	1988	369	1091	J BIOL CHEM HOPPESEYLE
ESSENBERG M K	1971	93	1242	J AM CHEM SOC
FINKE R G	1984	54	1	COORDIN CHEM REV
FINLAY T H	1972	247	4197	J BIOL CHEM
FINLAY T H	1973	248	1285	J BIOL CHEM
FORAGE R G	1979	569	249	BIOCHIM BIOPHYS ACTA
FORAGE R G	1982	149	413	J BACTERIOL
FORAGE R G	1982	151	591	J BACTERIOL
FREY P A	1967	29	873	BIOCHEM BIOPH RES CO
FREY P A	1970	92	4488	J AM CHEM SOC
FREY P A	1966	241	2732	J BIOL CHEM
FREY P A	1967	242	5369	J BIOL CHEM
GOLDING B T	1982	1	543	B12
HALPERN J	1984	106	8317	J AM CHEM SOC
HARTMANIS M G N	1986	245	144	JARCH BIOCHEM BIOPHYS
HAY B P	1986	108	4820	J AM CHEM SOC
HAY B P	1987	109	8012	J AM CHEM SOC
HONDA S	1980	143	1458	J BACTERIOL
HOSOI N	1978	56	566	J FERMENT TECHNOL
ICHIKAWA M	1988	952	191	BIOCHIM BIOPHYS ACTA
ISHIDA A	1993	32	1535	BIOCHEMISTRY-US
ISHIDA A	1993	39	115	J NUTR SCI VITAMINOL
JENSEN F R	1975	62	816	BIOCHEM BIOPH RES CO
JOHNSON B C	1975	42	315	METHOD ENZYMOL
KROUWER J S	1980	612	153	BIOCHIM BIOPHYS ACTA
KUNO S	1980	205	240	JARCH BIOCHEM BIOPHYS
KUNO S	1981	210	474	JARCH BIOCHEM BIOPHYS
KUNO S	1981	211	722	JARCH BIOCHEM BIOPHYS
KUNO S	1990	277	211	JARCH BIOCHEM BIOPHYS
LEE H A	1963	238	2367	J BIOL CHEM
MC GEE D E	1982	108	547	BIOCHEM BIOPH RES CO
MC GEE D E	1981	20	4293	BIOCHEMISTRY-US
MOORE K W	1979	87	1052	BIOCHEM BIOPH RES CO
OBRA DORS N	1988	170	2159	J BACTERIOL
OCHIAI E I	1975	37	351	J INORG NUCL CHEM
PAWELKIEWICZ J	1965	12	207	JACTA BIOCHIM POL
PAWELKIEWICZ J	1964	112	703	JANN NY ACAD SCI
POZNANSKAJA A A	1979	194	579	JARCH BIOCHEM BIOPHYS
POZNANSKAJA A A	1977	484	236	BIOCHIM BIOPHYS ACTA
QUASTEL J H	1925	19	304	BIOCHEM J
RETEY J	1966	22	502	JEXPERIENTIA
RETEY J	1966	22	72	JEXPERIENTIA
SCHLEPLER K L	1975	397	510	BIOCHIM BIOPHYS ACTA
SCHNEIDER Z	1966	114	7	JB ACAD POL SCI
SCHNEIDER Z	1970	245	3388	J BIOL CHEM
SCHUTZ H	1984	139	366	JARCH MICROBIOL
SMILEY K L	1962	97	538	JARCH BIOCHEM BIOPHYS
SPRENGER G A	1989	135	1255	J GEN MICROBIOL <-
STROINSKI A	1974	162	321	JARCH BIOCHEM BIOPHYS
STROINSKI A	1979	1	1029	VITAMIN B12
TALARICO T L	1990	56	1195	JAPPL ENVIRON MICROB
TANIZAWA K	1987	42	353	JZ NATURFORSCH C
TOBIMATSU T				JUNPUB
TONG I T	1991	57	3541	JAPPL ENVIRON MICROB
TORAYA T	1980	203	174	JARCH BIOCHEM BIOPHYS
TORAYA T	1985	242	470	JARCH BIOCHEM BIOPHYS
TORAYA T	1976	69	475	BIOCHEM BIOPH RES CO

TORAYA T 1971 10 3475 BIOCHEMISTRY-US  
 TORAYA T 1974 13 3895 BIOCHEMISTRY-US  
 TORAYA T 1975 14 3949 BIOCHEMISTRY-US  
 TORAYA T 1979 18 4417 BIOCHEMISTRY-US  
 TORAYA T 1988 27 7677 BIOCHEMISTRY-US  
 TORAYA T 1972 284 536 BIOCHIM BIOPHYS ACTA  
 TORAYA T 1982 2 233 512  
 TORAYA T 1977 76 285 EUR J BIOCHEM  
 TORAYA T 1978 135 726 J BACTERIOL  
 TORAYA T 1979 139 39 J BACTERIOL  
 TORAYA T 1980 141 1439 J BACTERIOL  
 TORAYA T 1977 252 963 J BIOL CHEM  
 TORAYA T 1980 255 3520 J BIOL CHEM  
 TORAYA T 1983 258 9296 J BIOL CHEM  
 TORAYA T 1986 261 9289 J BIOL CHEM  
 TORAYA T 1987 262 8544 J BIOL CHEM  
 TORAYA T 1991 266 5430 J BIOL CHEM  
 TORAYA T 1984 788 318 BIOCHIM BIOPHYS ACTA  
 USHIO K 1982 28 225 J NUTR SCI VITAMINOL  
 VALINSKY J E 1974 96 4709 J AM CHEM SOC  
 VALINSKY J E 1974 249 2751 J BIOL CHEM  
 VOISENET C E 1918 32 476 JANN I PASTEUR  
 WAGNER O W 1966 241 1751 J BIOL CHEM  
 WILLETT S A 1979 588 302 BIOCHIM BIOPHYS ACTA  
 YAKUSHEVA M I 1974 60 293 J ANAL BIOCHEM  
 YAKUSHEVA M I 1977 484 216 BIOCHIM BIOPHYS ACTA  
 YAMANE T 1966 113 362 J ARCH BIOCHEM BIOPHYS  
 ZAGALAK B 1964 11 49 J ACTA BIOCHIM POL  
 ZAGALAK B 1965 12 103 J ACTA BIOCHIM POL  
 ZAGALAK B 1965 12 219 J ACTA BIOCHIM POL  
 ZAGALAK B 1968 16 67 J B ACAD POL SCI  
 ZAGALAK B 1966 241 3028 J BIOL CHEM

L20 ANSWER 8 OF 9 SCISEARCH COPYRIGHT 1997 ISI (R)

AN 93:17810 SCISEARCH

GA The Genuine Article (R) Number: KE540

TI GROWTH TEMPERATURE-DEPENDENT ACTIVITY OF GLYCEROL DEHYDRATASE IN ESCHERICHIA-COLI EXPRESSING THE CITROBACTER-FREUNDII DHA REGULON

AU DANIEL R; GOTTSCHE G (Reprint)

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SO FEMS MICROBIOLOGY LETTERS, (15 DEC 1992) Vol. 100, No. 1-3, pp. 281-285. ISSN: 0378-1097. DT Article; Journal FS LIFE LA ENGLISH REC Reference Count: 13

AB Using the cosmid pWE15, a genomic library of Citrobacter freundii DNA in Escherichia coli ECL707 was prepared and screened for glycerol utilization. Six out of approximately 3000 clones were positive. One clone, harboring the recombinant cosmid pPD1, expressed glycerol dehydratase in high activity when grown at 28-degrees-C but not at 37-degrees-C. The growth temperature had little effect on the activity of the other enzymes encoded by the dha regulon. When the glycerol-containing medium was supplemented with cominoids, the recombinant E. coli strain produced 1,3-propanediol in high amounts at 28-degrees-C.

CC MICROBIOLOGY

ST Author Keywords: CITROBACTER-FREUNDII; ESCHERICHIA-COLI ECL707; GLYCEROL DEHYDRATASE; 1,3-PROPANEDIOL; GLYCEROL FERMENTATION; DHA REGULON

STP KeyWords Plus (R): KLEBSIELLA-PNEUMONIAE; ANAEROBIC GROWTH; GENES

RF 92-3056 001; UPTAKE OF SURFACTANT PROTEIN-B; CASEIN KINASE-II; CATALYTIC SUBUNITS

92-4812 001; PUTATIVE ANAEROBIC COPROPORPHYRINOGEN-III OXIDASE IN RHODOBACTER-SPHAEROIDES; TRANSCRIPTIONAL REGULATORY ELEMENT; FUNCTIONAL EXPRESSION

RE

Referenced Author	Year	VOL	PG	Referenced Work
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TORAYA T	1977	76	285	EUR J BIOCHEM
TORAYA T	1977	252	963	J BIOL CHEM

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AN 91:670800 SCISEARCH

GA The Genuine Article (R) Number: GT942

TI 1,3-PROPANEDIOL PRODUCTION BY ESCHERICHIA-COLI EXPRESSING GENES FROM THE KLEBSIELLA-PNEUMONIAE-DHA REGULON

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SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1991) Vol. 57, No. 12, pp. 3541-3546. DT Article; Journal FS LIFE; AGRILA ENGLISH REC Reference Count: 33

AB The dha regulon in Klebsiella pneumoniae enables the organism to grow anaerobically on glycerol and produce 1,3-propanediol (1,3-PD). Escherichia coli, which does not have a dha system, is unable to grow anaerobically on glycerol without an exogenous electron acceptor and does not produce 1,3-PD. A genomic library of K. pneumoniae ATCC 25955 constructed in E. coli AG1 was enriched for the ability to grow anaerobically on glycerol and dihydroxyacetone and was screened for the production of 1,3-PD. The cosmid pTC1 (42.5 kb total with an 18.2-kb major insert) was isolated from a 1,3-PD-producing strain of E. coli and found to possess enzymatic activities associated with four genes of the dha regulon: glycerol dehydratase (dhaB), 1,3-PD oxidoreductase (dhaT), glycerol dehydrogenase (dhaD), and dihydroxyacetone kinase (dhaK). All four activities were inducible by the presence of glycerol. When E. coli AG1/pTC1 was grown on complex medium plus glycerol, the yield of 1,3-PD from glycerol was 0.46 mol/mol. The major fermentation by-products were formate, acetate, and D-lactate. 1,3-PD is an intermediate in organic synthesis and polymer production. The 1,3-PD fermentation provides a useful model system for studying the interaction of a biochemical pathway in a foreign host and for developing strategies for metabolic pathway engineering.

CC MICROBIOLOGY; BIOTECHNOLOGY & APPLIED MICROBIOLOGY

STP KeyWords Plus (R): GLYCEROL; DISSIMILATION; DEHYDRATASES; COENZYME; KINASE

RF 91-1515 001; PHYSICAL MAP OF THE ESCHERICHIA-COLI CHROMOSOME; METZ GENE ENCODING TRANSFER-RNA MET F1; ASC (FORMERLY SAC) OPERON

RE

Referenced Author	Year	VOL	PG	Referenced Work
(RAU)		(RPL)	(RPG)	(RWK)

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BAILEY J E	1991	252	1668	SCIENCE
CAMERON D C	1990	1	1	J P CORN UT C ST LOU
COZZARELLI N R	1965	90	1325	J BACTERIOL
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ELM R	1980	19	425	J LULLMANNS ENCY TECHN
FORAGE R G	1979	569	249	BIOCHIM BIOPHYS ACTA
FORAGE R G	1982	149	413	J BACTERIOL
FORAGE R G	1982	151	591	J BACTERIOL
HONDA S	1980	143	1458	J BACTERIOL
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## DIALOG INFORMATION SERVICES

09dec97 12:47:19 User208600 Session D1120.1

File 301:CHEMNAME(R) 1957-1997/Nov (c) 1997 Amer.Chem.Soc.

S1 1 GLYCEROL(W)DEHYDRATASE  
S2 1 DIOL(W)DEHYDRATASE

## SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1966-1997/Dec W4 (c) format only 1997 Knight-Ridder Info  
File 5:BIOSIS PREVIEW(S) 1969-1997/Dec W1 (c) 1997 BIOSIS  
File 73:EMBASE 1974-1997/Nov W3 (c) 1997 Elsevier Science B.V.  
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## Set Items Description

S1 240 ADENOSYLCOBALAMIN()DEPENDENT()DIOL()DEHYDRASE + COENZYME() B12()DEPENDENT()DIOL()DEHYDRASE + COENZYME()B12()DEPENDENT()DIOL()DEHYDRATASE  
+ DEHYDRATASE()DIOL + DIOL()DEHYDRASE + DIOL()DEHYDRATASE + MESO()2()3()BUTANEDIOL()DEHYDRASE  
S2 117 PROPANEDIOL()DEHYDRASE + PROPANEDIOL()DEHYDRATASE + 1()2()PROPANEDIOL()DEHYDRATASE  
S3 280 S1:S2  
S4 191 COENZYME()B12()DEPENDENT()GLYCEROL()DEHYDRATASE + GLYCEROL()DEHYDRASE + GLYCEROL()DEHYDRATASE  
S5 156571 KLEBSIELLA OR CITROBACTER OR LACTOBACILLUS OR ENTEROBACTER OR PELOBACTER OR ILIOBACTER OR CLOSTRIDIUM  
S6 138 S3 AND S5  
S7 105 RD (unique items)  
S8 90 S4 AND S5 NOT S6  
S9 64 RD (unique items)

7/6/1 (Item 1 from file: 155) 08142159 97296406

Kinetic investigations with inhibitors that mimic the posthomolysis intermediate in the reactions of coenzyme-B12-dependent glycerol dehydratase and diol dehydratase.

7/6/2 (Item 2 from file: 155) 08960494 97157051

An electron paramagnetic resonance study on the mechanism-based inactivation of adenosylcobalamin-dependent diol dehydratase by glycerol and other substrates.

7/6/3 (Item 3 from file: 155) 08791004 96394290

Cloning, sequencing, and high level expression of the genes encoding adenosylcobalamin-dependent glycerol dehydratase of *Klebsiella pneumoniae*.

7/6/4 (Item 4 from file: 155) 08790962 96422012

Cloning, sequencing, and overexpression of the genes encoding coenzyme B12-dependent glycerol dehydratase of *Citrobacter freundii*.

7/6/5 (Item 5 from file: 155) 08213743 95221362

Molecular cloning, sequencing, and expression of the genes encoding adenosylcobalamin-dependent diol dehydratase of *Klebsiella oxytoca*.

7/6/6 (Item 6 from file: 155) 07662694 94015511

Importance of the nucleotide loop moiety coordinated to the cobalt atom of adenosylcobalamin for coenzymic function in the diol dehydratase reaction.

7/6/7 (Item 7 from file: 155) 07487018 93160191

Adenosylcobinamide methyl phosphate as a pseudocoenzyme for diol dehydratase.

7/6/8 (Item 8 from file: 155) 06454075 90165470

Essential histidine residues in coenzyme B12-dependent diol dehydratase: dye-sensitized photooxidation and ethoxycarbonylation.

7/6/9 (Item 9 from file: 155) 06216664 87092400

Solubilization of a membrane-bound diol dehydratase with retention of EPR  $g = 2.02$  signal by using 2-(N-cyclohexylamino)ethanesulfonic acid buffer.

7/6/10 (Item 10 from file: 155) 06148838 87265998

Re-investigation of the protein structure of coenzyme B12-dependent diol dehydratase.

7/6/11 (Item 11 from file: 155) 06130099 86129441

Diol metabolism and diol dehydratase in *Clostridium glycolicum*.

7/6/12 (Item 12 from file: 155) 06086412 88107822

Roles of the beta-D-ribofuranose ring and the functional groups of the D-ribose moiety of adenosylcobalamin in the diol dehydratase reaction.

7/6/13 (Item 13 from file: 155) 05575726 89207091

[Studies on the biological function of the nucleotide base of vitamin B12] Untersuchungen zur biologischen Funktion der Nucleotidbase von Vitamin B12.

7/6/14 (Item 14 from file: 155) 05554022 88198006

Anaerobic metabolism of the L-rhamnose fermentation product 1,2-propanediol in *Salmonella typhimurium*.

7/6/15 (Item 15 from file: 155) 05314924 87250467

Activation and cleavage of the carbon-cobalt bond of adenine-ethylcobalamin by diol dehydratase.

7/6/16 (Item 16 from file: 155) 05279470 86250875

The synthesis of adenine-modified analogs of adenosylcobalamin and their coenzymic function in the reaction catalyzed by diol dehydratase.

7/6/17 (Item 17 from file: 155) 04855866 86049396

The binding site for the adenosyl group of coenzyme B12 in diol dehydratase.

7/6/18 (Item 18 from file: 155) 04410929 80182104

The synthesis and properties of four spin-labeled analogs of adenosylcobalamin.

7/6/19 (Item 19 from file: 155) 03879299 82066866

Chemical modification of coenzyme B12-dependent diol dehydratase with pyridoxal 5'-phosphate: lysyl residue essential for interaction between two components of the enzyme.

7/6/20 (Item 20 from file: 155) 03841000 83074700

Diol dehydratase: N-terminal amino acid sequences and subunit stoichiometry.

7/6/21 (Item 21 from file: 155) 03837227 83032742

The mechanism of *in situ* reactivation of glycerol-inactivated coenzyme B12-dependent enzymes, glycerol dehydratase and diol dehydratase.

7/6/22 (Item 22 from file: 155) 03818946 82119943

Glycerol fermentation in *Klebsiella pneumoniae*: functions of the coenzyme B12-dependent glycerol and diol dehydratases.

7/6/23 (Item 23 from file: 155) 03817061 82099691

[The molecular basis of manifestation of function for vitamin B12 coenzymes (author's transl)]

7/6/24 (Item 24 from file: 155) 03814098 82066743

Reactive sulfhydryl groups of coenzyme B12-dependent diol dehydratase: differential modification of essential and nonessential ones.

7/6/25 (Item 25 from file: 155) 03810110 82023979

Purification and subunit characterization of propanediol dehydratase, a membrane-associated enzyme.

7/6/26 (Item 26 from file: 155) 03790172 81085020

Coenzyme B12-dependent diol dehydratase: chemical modification with 2,3-butanedione and phenylglyoxal.

7/6/27 (Item 27 from file: 155) 03783569 81006730

In situ reactivation of glycerol-inactivated coenzyme B12-dependent enzymes, glycerol dehydratase and diol dehydratase.

7/6/28 (Item 28 from file: 155) 03782938 80264192

Inactivation of diol dehydratase in the presence of a coenzyme-B12 analog.

7/6/29 (Item 29 from file: 155) 03775514 80159971

The synthesis of several immobilized derivatives of vitamin B12 coenzyme and their use as affinity adsorbents for a study of interactions of diol dehydratase with the coenzyme.

7/6/30 (Item 30 from file: 155) 03775503 80159893

Distribution of coenzyme B12-dependent diol dehydratase and glycerol dehydratase in selected genera of Enterobacteriaceae and Propionibacteriaceae.

7/6/31 (Item 31 from file: 155) 03260264 79231445

Stereospecificity and mechanism of adenosylcobalamin-dependent diol dehydratase. Catalysis and inactivation with meso- and dl-2,3-butanediols as substrates.

7/6/32 (Item 32 from file: 155) 03115235 79124674

Role of peripheral side chains of vitamin B12 coenzymes in the reaction catalyzed by diol dehydratase.

7/6/33 (Item 33 from file: 155) 03108208 78242158

Coenzyme B12-dependent diol dehydratase: regulation of apoenzyme synthesis in *Klebsiella pneumoniae* (*Aerobacter aerogenes*) ATCC 8724.

7/6/34 (Item 34 from file: 155) 02985254 77225263

Immunochemical evidence for the difference between coenzyme-B12-dependent diol dehydratase and glycerol dehydratase.

7/6/35 (Item 35 from file: 155) 02963505 80000580

Resolution of the coenzyme B-12-dependent dehydratases of *Klebsiella* sp. and *Citrobacter freundii*.

7/6/36 (Item 36 from file: 155) 02963490 80000417

Hydrogen transfer in catalysis by adenosylcobalamin-dependent diol dehydratase.

7/6/37 (Item 37 from file: 155) 02959767 79216215

Fermentation of 1,2-propanediol with 1,2-ethanediol by some genera of Enterobacteriaceae, involving coenzyme B12-dependent diol dehydratase.

7/6/38 (Item 38 from file: 155) 02956999 79186157

Coenzyme B12-dependent diol dehydratase: purification, subunit heterogeneity, and reversible association.

7/6/39 (Item 39 from file: 155) 02908486 77134713

Mechanism of action of adenosylcobalamin: glycerol and other substrate analogues as substrates and inactivators for propanediol dehydratase—kinetics, stereospecificity, and mechanism.

7/6/40 (Item 40 from file: 155) 02907797 77118572

Studies on the mechanism of the adenosylcobalamin-dependent diol dehydratase reaction by the use of analogs of the coenzyme.

7/6/41 (Item 41 from file: 155) 02313562 75146954

Preparation, properties and biological activities of succinyl derivatives of vitamin B12.

7/6/42 (Item 42 from file: 155) 02143923 76184142

Substrate specificity of coenzyme B12-dependent diol dehydratase: glycerol as both a good substrate and a potent inactivator.

7/6/43 (Item 43 from file: 155) 02058873 76039896

Immobilized diol dehydratase and its use in studies of cobalamin binding and subunit interaction.

7/6/44 (Item 44 from file: 155) 02002592 75146949

Ethanolamine ammonia-lyase: inactivation of the holoenzyme by N<sub>2</sub>O and the mechanism of action of Coenzyme B12.

7/6/45 (Item 45 from file: 155) 01430076 75008121

Coenzyme B12 dependent diol dehydratase system. Dissociation of the enzyme into two different protein components and some properties of the components.

7/6/46 (Item 46 from file: 155) 01317779 74031427

Activation of diol dehydratase by formamidineium or guanidineium ion, polyatomic monovalent cations having sp<sup>2</sup> nitrogen.

7/6/47 (Item 47 from file: 155) 01276156 73196460

Dissociation of diol dehydratase into two different protein components.

7/6/48 (Item 48 from file: 155) 01201378 73047392

Coenzyme B12-dependent propanediol dehydratase systems. Ternary complex between apoenzyme, coenzyme, and substrate analog.

7/6/49 (Item 49 from file: 155) 01158359 72238147

Coenzyme B12 dependent propanediol dehydratase system. Nature of cobalamin binding and some properties of apoenzyme-coenzyme B12 analog complexes.

7/6/50 (Item 50 from file: 155) 00961699 72040213

Propanediol dehydratase system. Role of monovalent cations in binding of vitamin B12 coenzyme or its analogs to apoenzyme.

7/6/51 (Item 51 from file: 155) 00495878 70000235

Ternary complex formation of 1,2-propanediol dehydratase, cobamide coenzyme and substrate analogue.

7/6/52 (Item 52 from file: 155) 00227137 68011874

Coenzyme activity of 5'-chlorocobalamin (10-Cl-DBCC) in propanediol dehydratase system.

7/6/53 (Item 53 from file: 155) 00143034 67173019

[On the mechanism of the propanediol dehydratase reaction] Zum Mechanismus der Propandioldehydratase-Reaktion.

7/6/54 (Item 54 from file: 155) 00102610 67052680

[On the stereochemistry of the propanediol dehydratase reaction] Zur Stereochemie der Propandioldehydratase-Reaktion.

7/6/55 (Item 1 from file: 5) 13011721 BIOSIS Number: 99011721

Carbon and electron flow in *Clostridium butyricum* grown in chemostat culture on glycerol and on glucose Print Number: Biological Abstracts Vol. 102ss. 001 Ref. 011721

7/6/56 (Item 2 from file: 5) 11049541 BIOSIS Number: 97249541

Diol dehydratase and glycerol dehydratase, coenzyme B-12-dependent isozymes Print Number: Biological Abstracts/RRM Vol. 048ss. 006 Ref. 082721

7/6/57 (Item 3 from file: 5) 11049540 BIOSIS Number: 97249540

Diol dehydratase from *Clostridium glycolicum*: The non-B-12-dependent enzyme Print Number: Biological Abstracts/RRM Vol. 048ss. 006 Ref. 082720

7/6/58 (Item 4 from file: 5) 11049533 BIOSIS Number: 97249533

Metal Ions in Biological Systems, Vol. 30 Metalloenzymes involving amino acid-residue and related radicals Print Number: Biological Abstracts/RRM Vol. 046ss. 006 Ref. 082713

7/6/59 (Item 5 from file: 5) 9567450 BIOSIS Number: 94072450

ENZYMES INVOLVED IN ANAEROBIC POLYETHYLENE GLYCOL DEGRADATION BY PELOBACTER-VENETIANUS AND BACTEROIDES STRAIN PG1

7/6/60 (Item 6 from file: 5) 5816885 BIOSIS Number: 83079192

SOLUBILIZATION OF A MEMBRANE-BOUND DIOL DEHYDRATASE WITH RETENTION OF EPR G EQUALS 2.02 SIGNAL BY USING 2-N CYCLOHEXYLAMINOETHANESULFONIC ACID BUFFER

7/6/61 (Item 7 from file: 5) 5447256 BIOSIS Number: 82092059

CHARACTERIZATION OF THE ENZYME INVOLVED IN FORMATION OF 2 BUTANOL FROM MESO-2,3 BUTANEDIOL BY LACTIC-ACID BACTERIA

7/6/62 (Item 8 from file: 5) 5150605 BIOSIS Number: 31039920  
SOLUBILIZATION OF MEMBRANE-BOUND AND OXYGEN SENSITIVE ENZYMES WITH 2-N CYCLOHEXYLAMINOETHANESULFONIC-ACID

7/6/63 (Item 9 from file: 5) 5104752 BIOSIS Number: 30117059  
SOLUBILIZATION OF DIOL DEHYDRATASE FROM CLOSTRIDIUM-GLYCOLICUM

7/6/64 (Item 10 from file: 5) 4792137 BIOSIS Number: 79034452  
COENZYMIC FUNCTION OF 1 SUBSTITUTED OR N-6 SUBSTITUTED ANALOGS OF ADENOSYLCOBALAMIN IN THE DIOL DEHYDRATASE EC-4.2.1.28 REACTION

7/6/65 (Item 11 from file: 5) 4650225 BIOSIS Number: 29007540  
DIOL DEHYDRATASE AND GLYCOL METABOLISM IN CLOSTRIDIUM-GLYCOLICUM

7/6/66 (Item 12 from file: 5) 4440303 BIOSIS Number: 78014126  
LIGAND EXCHANGE REACTIONS OF DIOL DEHYDRASE EC-4.2.1.28 BOUND COBALAMINS AND THE EFFECT OF THE NUCLEOSIDE BINDING

7/6/67 (Item 13 from file: 5) 4071486 BIOSIS Number: 76021337  
DIOL DEHYDRATASE EC-4.2.1.28 N TERMINAL AMINO-ACID SEQUENCES AND SUBUNIT STOICHIOMETRY

7/6/68 (Item 14 from file: 5) 4027710 BIOSIS Number: 75075069  
THE MECHANISM OF IN-SITU REACTIVATION OF GLYCEROL INACTIVATED COENZYME B-12 DEPENDENT ENZYMES GLYCEROL DEHYDRATASE EC-4.2.1.30 AND DIOL DEHYDRATASE EC-4.2.1.28

7/6/69 (Item 15 from file: 5) 3664154 BIOSIS Number: 73056521  
REACTIVE SULFHYDRYL GROUPS OF COENZYME B-12 DEPENDENT DIOL DEHYDRASE EC-4.2.1.28 DIFFERENTIAL MODIFICATION OF ESSENTIAL AND NONESSENTIAL ONES

7/6/70 (Item 16 from file: 5) 3642292 BIOSIS Number: 73034659  
PURIFICATION AND SUBUNIT CHARACTERIZATION OF PROPANEDIOL DEHYDRATASE EC-4.2.1.28 A MEMBRANE ASSOCIATED ENZYME

7/6/71 (Item 17 from file: 5) 3318561 BIOSIS Number: 71040960  
COENZYME B-12 DEPENDENT DIOL DEHYDRASE EC-4.2.1.28 CHEMICAL MODIFICATION WITH 2,3-BUTANEDIONE AND PHENYL GLYOXAL

7/6/72 (Item 18 from file: 5) 3237789 BIOSIS Number: 21030192  
STRUCTURE FUNCTION RELATIONSHIP OF VITAMIN B-12 COENZYME ADENOSYL COBALAMIN IN THE DIOL DEHYDRASE EC-4.2.1.28 SYSTEM

7/6/73 (Item 19 from file: 5) 3086882 BIOSIS Number: 70036789  
THE SYNTHESIS OF SEVERAL IMMOBILIZED DERIVATIVES OF VITAMIN B-12 COENZYME AND THEIR USE AS AFFINITY ADSORBENTS FOR A STUDY OF INTERACTIONS OF DIOL DEHYDRASE EC-4.2.1.28 WITH THE COENZYME

7/6/74 (Item 20 from file: 5) 2974674 BIOSIS Number: 69012081  
FERMENTATION OF 1,2-PROPANEDIOL AND 1,2-ETHANEDIOL BY SOME GENERA OF ENTEROBACTERIACEAE INVOLVING COENZYME B-12 DEPENDENT DIOL DEHYDRATASE EC-4.2.1.28

7/6/75 (Item 21 from file: 5) 2801475 BIOSIS Number: 68056382  
COENZYME B-12 DEPENDENT DIOL DEHYDRASE EC-4.2.1.28 PURIFICATION SUBUNIT HETEROGENEITY AND REVERSIBLE ASSOCIATION

7/6/76 (Item 22 from file: 5) 2788680 BIOSIS Number: 68043587  
STEREOSPECIFICITY AND MECHANISM OF ADENOSYL COBALAMIN DEPENDENT DIOL DEHYDRATASE CATALYSIS AND INACTIVATION WITH MESO-2,3-BUTANEDIOL AND RACEMIC 2,3-BUTANEDIOL AS SUBSTRATES

7/6/77 (Item 23 from file: 5) 2756523 BIOSIS Number: 68011430  
ROLE OF PERIPHERAL SIDE CHAINS OF VITAMIN B-12 COENZYMES IN THE REACTION CATALYZED BY DIOL DEHYDRASE EC-4.2.1.28

7/6/78 (Item 24 from file: 5) 2684235 BIOSIS Number: 67021638  
METABOLISM OF 1,2-PROPANEDIOL BY METHANOL UTILIZING BACTERIA AND SOME PROPERTIES OF 1,2-PROPANEDIOL DEHYDROGENATING ENZYME

7/6/79 (Item 25 from file: 5) 2526149 BIOSIS Number: 66073054  
COENZYME B-12 DEPENDENT DIOL DEHYDRATASE EC-4.2.1.28 REGULATION OF APOENZYME SYNTHESIS IN KLEBSIELLA-PNEUMONIAE AEROBACTER-AEROGENES ATCC-8724

7/6/80 (Item 26 from file: 5) 2501151 BIOSIS Number: 66048056  
MECHANISM OF ACTION OF ADENOSYL COBALAMIN HYDROGEN TRANSFER IN THE INACTIVATION OF DIOL DEHYDRATASE EC-4.2.1.28 BY GLYCEROL

7/6/81 (Item 27 from file: 5) 2377808 BIOSIS Number: 65004216  
IMMUNOCHEMICAL EVIDENCE FOR THE DIFFERENCE BETWEEN COENZYME B-12 DEPENDENT DIOL DEHYDRATASE EC-4.2.1.28 AND GLYCEROL DEHYDRATASE EC-4.2.1.30

7/6/82 (Item 28 from file: 5) 2183678 BIOSIS Number: 64010598  
MECHANISM OF ACTION OF ADENOSYL COBALAMIN GLYCEROL AND OTHER SUBSTRATE ANALOGS AS SUBSTRATES AND INACTIVATORS FOR PROPANEDIOL DEHYDRATASE EC-4.2.1.28 KINETICS STEREOSPECIFICITY AND MECHANISM

7/6/83 (Item 29 from file: 5) 2166587 BIOSIS Number: 63071007  
STUDIES ON THE MECHANISM OF THE ADENOSYL COBALAMIN DEPENDENT DIOL DEHYDRASE EC-4.2.1.28 REACTION BY THE USE OF ANALOGS OF THE COENZYME

7/6/84 (Item 30 from file: 5) 1721539 BIOSIS Number: 60066107  
A PHYSICAL EXPLANATION OF THE EPR SPECTRUM OBSERVED DURING CATALYSIS BY ENZYMES UTILIZING COENZYME B-12

7/6/85 (Item 31 from file: 5) 1677514 BIOSIS Number: 60022082  
ETHANOL AMINE AMMONIA LYASE INACTIVATION OF THE HOLO ENZYME BY NITROGEN OXIDE AND THE MECHANISM OF ACTION OF COENZYME B-12

7/6/86 (Item 32 from file: 5) 1671435 BIOSIS Number: 60016003  
RELATIVE ENANTIOMER BINDING AND REACTION RATES WITH PROPANEDIOL DEHYDRASE EC-4.2.1.28

7/6/87 (Item 33 from file: 5) 1083898 BIOSIS Number: 55013830  
FORMATION OF 5-DEOXYADENOSYL DERIVATES OF COBALAMIN C LACTAM AND COBALAMIN C LACTONE BY PROPIONIBACTERIUM-SHERMANII IN-VIVO AND IN-VITRO

7/6/88 (Item 1 from file: 73) 10002807 EMBASE No: 96181477  
Evidence for enantiomorphic-enantiotopic group discrimination in diol dehydratase-catalyzed dehydration of meso-2,3-butanediol

7/6/89 (Item 2 from file: 73) 9133324 EMBASE No: 94072716  
The synthesis of a pyridyl analog of adenosylcobalamin and its coenzymic function in the diol dehydratase reaction

7/6/90 (Item 3 from file: 73) 8280211 EMBASE No: 91302965  
Roles of the D-ribose and 5,6-dimethylbenzimidazole moieties of the nucleotide loop of adenosylcobalamin in manifestation of coenzymic function in the diol dehydratase reaction

7/6/91 (Item 4 from file: 73) 7247646 EMBASE No: 88247524  
Acceleration of cleavage of the carbon-cobalt bond of sterically hindered alkylcobalamins by binding to a protein of diol dehydratase

7/6/92 (Item 5 from file: 73) 6031502 EMBASE No: 86026562  
The binding site for the adenosyl group of coenzyme B<sub>12</sub> in diol dehydratase

7/6/93 (Item 6 from file: 73) 5754272 EMBASE No: 84249938  
Propanediol-1,2-dehydratase and metabolism of glycerol of Lactobacillus brevis

7/6/94 (Item 7 from file: 73) 5710823 EMBASE No: 84206489  
Coenzymic function of 1- or N<sub>6</sub>-substituted analogs of adenosylcobalamin in the diol dehydratase reaction

7/6/95 (Item 8 from file: 73) 5125653 EMBASE No: 82130576  
Glycerol fermentation in Klebsiella pneumoniae: Functions of the coenzyme B<sub>12</sub> subunit 2-dependent glycerol and diol dehydratases

7/6/96 (Item 9 from file: 73) 2260075 EMBASE No: 81031200  
In situ reactivation of glycerol-inactivated coenzyme B<sub>12</sub> subunit 2-dependent enzymes, glycerol dehydratase and diol dehydratase

7/6/97 (Item 10 from file: 73) 1232941 EMBASE No: 79000296

7/6/98 (Item 11 from file: 73) 949780 EMBASE No: 78117989

Immunochemical evidence for the difference between coenzyme Bsub 1sub 2 dependent diol dehydratase and glycerol dehydratase

7/6/99 (Item 12 from file: 73) 616302 EMBASE No: 76203083

Mechanism of action of adenosylcobalamin: 3fluoro 1,2 propanediol as substrate for propanediol dehydrase. Mechanistic implications

7/6/100 (Item 13 from file: 73) 556098 EMBASE No: 76140982

Coenzyme action of adenosyl 13 epicalamin in the diol dehydrase system

7/6/101 (Item 14 from file: 73) 537094 EMBASE No: 76121511

A physical explanation of the EPR spectrum observed during catalysis by enzymes utilizing coenzyme Bsub 1sub 2

7/6/102 (Item 15 from file: 73) 427247 EMBASE No: 76007141

Relative enantiomer binding and reaction rates with propanediol dehydrase

7/6/103 (Item 16 from file: 73) 326271 EMBASE No: 75119035

Coenzyme Bsub 1sub 2 dependent diol dehydrase system. Dissociation of the enzyme into two different protein components and some properties of the components

7/6/104 (Item 1 from file: 351) 011021737 WPIAcc No: 96-518687/199651

Fermentative prodn. of 1,3-propane-diol useful for polymer prodn. - from carbon substrates using mixed culture of glycerol-producing and diol-producing organisms

7/6/105 (Item 2 from file: 351) 011021733 WPIAcc No: 96-518683/199651

Cosmid contg. Klebsiella pneumoniae gene for diol dehydratase - and related transformed microorganisms able to convert glycerol to 1,3-propanediol for polymer prodn

7/7/3 (Item 3 from file: 155) DIALOG(R)File 155:MEDLINE(R) (c) format only 1997 Knight-Ridder Info. Allrts. reserv.

08791004 96394290

Cloning, sequencing, and high level expression of the genes encoding adenosylcobalamin-dependent glycerol dehydrase of Klebsiella pneumoniae.

Tobimatsu T; Azuma M; Matsubara H; Takatori H; Niida T; Nishimoto K; Satoh H; Hayashi R; Toraya T

Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Tsushima-Naka, Okayama 700, Japan.

J Biol Chem (UNITED STATES) Sep 13 1996, 271 (37) p22352-7, ISSN 0021-9258 Journal Code: HIV Languages: ENGLISH Document type: JOURNAL ARTICLE

The gld genes encoding adenosylcobalamin-dependent glycerol dehydrase of Klebsiella pneumoniae were cloned by cross-hybridization with a DNA fragment of Klebsiella oxytoca diol dehydrase genes. Since the Escherichia coli clones isolated did not show appreciable enzyme activity, plasmids for high level expression of cloned genes were constructed. The enzyme expressed in E. coli was indistinguishable from the wild-type glycerol dehydrase of K. pneumoniae by the criteria of polyacrylamide gelelectrophoretic, immunochemical, and catalytic properties. It was also shown that the recombinant functional enzyme consists of Mr 61,000, 22,000, and 16,000 subunits. Sequence analysis of the genes revealed four open reading frames separated by 2-12 bases. The sequential three open reading frames from the first to the third (gldA, gldB, and gldC genes) encoded polypeptides of 555, 194, and 141 amino acid residues with predicted molecular weights of 60,659(alpha), 21,355(beta), and 16,104(gamma), respectively. High level expression of these three genes in E. coli produced more than 14-fold higher level of fully active apoenzyme than that in K. pneumoniae. It was thus concluded that these are the genes encoding the subunits of glycerol dehydrase. The deduced amino acid sequences of the three subunits were 71, 58, and 54% identical with those of the alpha, beta, and gamma subunits of diol dehydrase, respectively, but failed to show any apparent homology with other proteins.

7/7/4 (Item 4 from file: 155) DIALOG(R)File 155:MEDLINE(R) (c) format only 1997 Knight-Ridder Info. Allrts. reserv.

08790962 96422012

Cloning, sequencing, and overexpression of the genes encoding coenzyme B12-dependent glycerol dehydratase of Citrobacter freundii.

Seyfried M; Daniel R; Gottschalk G

Institut für Mikrobiologie der Georg-August-Universität, Göttingen, Germany.

J Bacteriol (UNITED STATES) Oct 1996, 178 (19) p5793-6, ISSN 0021-9193 Journal Code: HH3 Languages: ENGLISH Document type: JOURNAL ARTICLE

The genes encoding coenzyme B12-dependent glycerol dehydratase of Citrobacter freundii were cloned and overexpressed in Escherichia coli. The B12-free enzyme was purified to homogeneity. It consists of three types of subunits whose N-terminal sequences are in accordance with those deduced from the open reading frames dhaB, dhaC, and dhaE, coding for subunits of 60,433 (alpha), 21,487 (beta), and 16,121 (gamma) Da, respectively. The enzyme complex has the composition alpha2beta2gamma2. Amino acid alignments with the subunits of the recently sequenced diol dehydratase of Klebsiella oxytoca (T. Tobimatsu, T. Hara, M. Sakaguchi, Y. Kishimoto, Y. Wada, M. Isoda, T. Sakai, and T. Toraya, J. Biol. Chem. 270:7142-7148, 1995) revealed identities between 51.8 and 70.9%.

7/7/5 (Item 5 from file: 155) DIALOG(R)File 155:MEDLINE(R) (c) format only 1997 Knight-Ridder Info. Allrts. reserv.

08213743 95221362

Molecular cloning, sequencing, and expression of the genes encoding adenosylcobalamin-dependent diol dehydrase of Klebsiella oxytoca.

Tobimatsu T; Hara T; Sakaguchi M; Kishimoto Y; Wada Y; Isoda M; Sakai T; Toraya T

Department of Biotechnology, Faculty of Engineering, Okayama University, Japan.

J Biol Chem (UNITED STATES) Mar 31 1995, 270 (13) p7142-8, ISSN 0021-9258 Journal Code: HIV Languages: ENGLISH Document type: JOURNAL ARTICLE

The pdd genes encoding adenosylcobalamin-dependent diol dehydrase of Klebsiella oxytoca were cloned by using a synthetic oligodeoxynucleotide as a hybridization probe followed by measuring the enzyme activity of each clone. Five clones of Escherichia coli exhibited diol dehydrase activity. At least one of them was shown to express diol dehydrase genes under control of their own promoter. Sequence analysis of the DNA fragments found in common in the inserts of these five clones and the flanking regions revealed four open reading frames separated by 10-18 base pairs. The sequential three open reading frames from the second to the fourth (pddA, pddB, and pddC genes) encoded polypeptides of 554, 224, and 173 amino acid residues with predicted molecular weights of 60,348 (alpha), 24,113 (beta), and 19,173 (gamma), respectively. Overexpression of these three genes in E. coli produced more than 50-fold higher level of functional apodiol dehydrase than that in K. oxytoca. The recombinant enzyme was indistinguishable from the wild-type one of K. oxytoca by the criteria of polyacrylamide gel electrophoretic and immunochemical properties. It was thus concluded that these three gene products are the subunits of functional diol dehydrase. Comparisons of the deduced amino acid sequences of the three subunits with other proteins failed to reveal any apparent homology.

7/7/11 (Item 11 from file: 155) DIALOG(R)File 155:MEDLINE(R) (c) format only 1997 Knight-Ridder Info. Allrts. reserv.

06130099 86129441

Diol metabolism and diol dehydratase in Clostridium glycolicum.

Hartmanis MG; Stadtman TC

Arch Biochem Biophys (UNITED STATES) Feb 15 1986, 245 (1) p144-52, ISSN 0003-9861 Journal Code: 6SK Languages: ENGLISH Document type: JOURNAL ARTICLE

Levels of the five enzymes involved in the fermentation of 1,2-ethanediol and 1,2-propanediol in the strictly anaerobic bacterium, Clostridium glycolicum, were investigated. All enzymes with the exception of the first enzyme in the pathway, diol dehydratase, were found to be constitutive, stable to exposure to oxygen, and present in the cytosol. Diol dehydratase was found to be extremely oxygen sensitive and strongly associated with the cell membrane. Treatment with ionic and nonionic detergents, butanol, phospholipase A2, or osmotic shock procedures failed to solubilize any diol dehydratase activity. Limited proteolysis using subtilisin released small amounts of activity. Diol dehydratase was found to be specific for 1,2-ethanediol and 1,2-propanediol and required the addition of a reducing agent for maximal activity. The enzyme was strongly inhibited by low concentrations of EDTA, ethylene glycol bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid, o-phenanthroline, hydroxylamine, hydroxyurea, and sulphydryl reagents. Addition of adenosylcobalamin or high levels of intrinsic factor did not affect the reaction rate. Irradiation with light also did not inhibit the enzyme activity. These results suggest that the catalytic mechanism of diol dehydratase from C. glycolicum does not involve a cobamide coenzyme.

7/7/22 (Item 22 from file: 155) DIALOG(R)File 155:MEDLINE(R) (c) format only 1997 Knight-Ridder Info. Allrts. reserv.

03818946 82119943

Glycerol fermentation in Klebsiella pneumoniae: functions of the coenzyme B12-dependent glycerol and diol dehydratases.

Forage RG; Foster MA

J Bacteriol (UNITED STATES) Feb 1982, 149 (2) p413-9, ISSN 0021-9193 Journal Code: HH3 Languages: ENGLISH Document type: JOURNAL ARTICLE

Glycerol and diol dehydratases are inducible, coenzyme B12-dependent enzymes found together in Klebsiella pneumoniae ATCC 25955 during anaerobic growth on glycerol. Mutants of this strain isolated by a novel procedure were separately constitutive for either dehydratase, showing the structural genes for the two enzymes to be under independent control in vivo. Glycerol dehydratase and a trimethylene glycol dehydrogenase were implicated as members of a pleiotropic control system that includes glycerol dehydrogenase and dihydroxyacetone kinase for the anaerobic dissimilation of glycerol (the "dha system"). The dehydratase and dehydrogenases were induced by dihydroxyacetone and were jointly constitutive in mutants isolated as constitutive for either the dha system or glycerol dehydratase. These data and the stimulation of growth by Co2+ suggested that glycerol dehydratase and trimethylene glycol dehydrogenase are obligatory enzymes for anaerobic growth on glycerol as the sole carbon source.

7/7/56 (Item 2 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

11049541 BIOSIS Number: 97249541

Diol dehydrase and glycerol dehydrase, coenzyme B-12-dependent isozymes

Toraya T

Dep. Biotechnol., Fac. Eng., Okayama Univ., 3-1-1 Tsushima-Naka, Okayama 700, JAP 0 (0), 1994. 217-254. Full Journal Title: Sigel, H. and A. Sigel (Ed.), Metal Ions in Biological Systems, Vol. 30. Metalloenzymes

involving amino acid-residue and related radicals. xxv+494p. Marcel Dekker, Inc.: New York, New York, USA; Basel, Switzerland. ISBN 0-8247-9093-6. ISSN: 0161-5149

Language: ENGLISH Print Number: Biological Abstracts/RM Vol. 046 Iss. 006 Ref. 082721

7/7/57 (Item 3 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.



7/7/58 (Item 4 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R)(c) 1997 BIOSIS. Allrts. reserv.

11049533 BIOSIS Number: 97249533

Metal Ions in Biological Systems, Vol. 30. Metalloenzymes involving amino acid-residue and related radicals

Sigel H; Sigel A

Inst. Inorg. Chem., Univ. Basel, CH-4056 Basel, SWI 0 (0). 1994. XXXV+494P. Full Journal Title: Sigel, H. and A. Sigel (Ed.). Metal Ions in Biological Systems, Vol. 30. Metalloenzymes involving amino acid-residue and related radicals. xxv+494p. Marcel Dekker, Inc.: New York, New York, USA; Basel, Switzerland. ISBN 0-8247-9093-6. ISSN: 0161-5149

Language: ENGLISH Print Number: Biological Abstracts/RRM Vol. 046 Iss. 006 Ref. 082713

This book contains 13 papers discussing metalloenzymes involving amino acid-residue and related radicals. Some of the topics covered include free radical sites and their locations, mechanistic considerations, and enzymes that depend on the metals manganese, iron, cobalt, and copper. The work will be useful for researchers and students in chemistry, biochemistry, biophysics, enzymology, molecular biology, etc. Graphs, diagrams, tables, and charts illustrate the text.

7/7/59 (Item 5 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R)(c) 1997 BIOSIS. Allrts. reserv.

9567450 BIOSIS Number: 94072450

ENZYMES INVOLVED IN ANAEROBIC POLYETHYLENE GLYCOL DEGRADATION BY PELOBACTER-VENETIANUS AND BACTEROIDES STRAIN PG1

FRINGS J; SCHRAMM E; SCHINK B

FAKULTÄT FÜR BIOLOGIE DER UNIVERSITÄT KONSTANZ, POSTFACH 5560, D-7750 KONSTANZ, GERMANY.

APPL ENVIRON MICROBIOL 58 (7). 1992. 2164-2167. CODEN: AEMID Full Journal Title: Applied and Environmental Microbiology Language: ENGLISH

In extracts of polyethylene glycol (PEG)-grown cells of the strictly anaerobically fermenting bacterium *Pelobacter venetianus*, two different enzyme activities were detected, a diol dehydratase and a PEG-degrading enzyme which was characterized as a PEG acetaldehyde lyase. Both enzymes were oxygen sensitive and depended on a reductant, such as titanium citrate or sulfhydryl compounds, for optimal activity. The diol dehydratase was inhibited by various corrinoids (adenosylcobalamin, cyanocobalamin, hydroxocobalamin, and methylcobalamin) by up to 37% at a concentration of 100  $\mu$ M. Changes in ionic strength and the K<sup>+</sup> ion concentration had only limited effects on this enzyme activity; glycerol inhibited the enzyme by 95%. The PEG-degrading enzyme activity was stimulated by the same corrinoids by up to 80%, exhibited optimal activity in 0.75 M potassium phosphate buffer or in the presence of 4 M KCl, and was only slightly affected by glycerol. Both enzymes were located in the cytoplasmic space. Also, another PEG-degrading bacterium, *Bacteroides* strain PG1, contained a PEG acetaldehyde lyase activity analogous to the corresponding enzyme of *P. venetianus* but no diol dehydratase. Our results confirm that corrinoid-influenced PEG degradation analogous to a diol dehydratase reaction is a common strategy among several different strictly anaerobic PEG-degrading bacteria.

7/7/74 (Item 20 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R)(c) 1997 BIOSIS. Allrts. reserv.

2974674 BIOSIS Number: 69012081

FERMENTATION OF 1,2-PROPANEDIOL AND 1,2-ETHANEDIOL BY SOME GENERA OF ENTEROBACTERIACEAE INVOLVING COENZYME B-12 DEPENDENT DIOL DEHYDRATASE EC-4.2.1.28

TORAYA T; HONDA S; FUKUI S

LAB. IND. BIOCHEM., DEP. IND. CHEM., FAC. ENG., KYOTO UNIV., SAKYO, KYOTO 606, JPN.

J BACTERIOL 139 (1). 1979. 39-47. CODEN: JOBAA Full Journal Title: Journal of Bacteriology Language: ENGLISH

*Klebsiella pneumoniae* (Aerobacter aerogenes) ATCC 8724 grew anaerobically on 1,2-propanediol and 1,2-ethanediol as C and energy sources. Whole cells of the bacterium grown anaerobically on 1,2-propanediol or on glycerol catalyzed conversion of 1,2-diols and aldehydes on the corresponding acids and alcohols. Glucose-grown cells also converted aldehydes, but not 1,2-diols, to acids and alcohols. The presence of activities of coenzyme B12-dependent diol dehydratase, alcohol dehydrogenase, Co-A-dependent aldehyde dehydrogenase, phosphotransacetylase and acetate kinase was demonstrated with crude extracts of 1,2-propanediol-grown cells. The dependence of the levels of these enzymes on growth substrates, together with cofactor requirements in *in vitro* conversion of these substrates, indicates that 1,2-diols are fermented to the corresponding acids and alcohols via aldehydes, acyl-CoA and acyl phosphates. This metabolic pathway for 1,2-diol fermentation was also suggested in some other genera of Enterobacteriaceae which grew anaerobically on 1,2-propanediol. When the bacteria were cultivated in a 1,2-propanediol medium not supplemented with cobalt ion, the coenzyme B12-dependent conversion of 1,2-diols to aldehydes was the rate-limiting step in this fermentation. This was because the intracellular concentration of coenzyme B12 was very low in the cells grown in cobalt-deficient medium, since the apoprotein of diol dehydratase was markedly induced in the cells grown in the 1,2-propanediol medium. Better cell yields were obtained when the bacteria were grown anaerobically on 1,2-propanediol. Aerobically grown cells evidently have a different metabolic pathway for utilizing 1,2-propanediol.

7/7/105 (Item 2 from file: 351) DIALOG(R)File 351:DERWENT WPI (c)1997 Derwent Info Ltd. Allrts. reserv.

011021733 WPI Acc No: 96-518683/199651

Cosmid contg. *Klebsiella pneumoniae* gene for diol dehydratase - and related transformed microorganisms able to convert glycerol to 1,3-propanediol for polymer prodn

Patent Assignee: DU PONT DE NEMOURS &amp; CO E I (DUPO )

Inventor: NAGARAJAN V; NAKAMURA C E

Number of Countries: 061 Number of Patents: 003

Patent Family:

Patent No Kind Date Applicat No Kind Date Main IPC Week

WO 9635795 A1 19961114 WO 96US6163 A 19960502 C12N-015/60 199651 B

AU 9657229 A 19961129 AU 9657229 A 19960502 C12N-015/60 199712

US 5633362 A 19970527 US 95440377 A 19950512 C07H-021/02 199727

Priority Applications (No Type Date): US 95440377 A 19950512

Cited Patents: 9, journal ref.

Patent Details:

Patent Kind Lan Pg Filing Notes Application Patent

WO 9635795 A1 E 48

Designated States (National): AL AU BB BG BR CA CN CZ EE GE HU IS JP KPRK LK LR LT LV MG MK MN MX NO NZ PL RO SG SI SK TR TT UA US UZ VN

Designated States (Regional): AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG

AU 9657229 A Based on WO 9635795

US 5633362 A 18

Abstract (Basic): WO 9635795 A

Cosmid (A) comprises a DNA fragment (I) of about 35 kb from *Klebsiella pneumoniae* that encodes an active diol dehydratase enzyme (II).

USE - Cells transformed with (I) or (A) can convert glycerol to 1,3-propanediol (IV) which is a monomer potentially useful for prodn. of polyester fibre, polyurethanes and cyclic cpds.

ADVANTAGE - This method provides efficient, cost effective and environmentally acceptable prodn. of (IV).

Dwg. 0/4

Abstract (Equivalent): US 5633362 A

A cosmid comprising a DNA fragment of about 35 kb isolated from *Klebsiella pneumoniae* wherein said fragment encodes an active diol dehydratase enzyme having the restriction digest in FIG. 5, columns numbered 4, said cosmid contained within a transformed *E. coli* deposited with the American Type Culture Collection under accession number ATCC 69790. (Fig 5 not suitable for reproduction)

Dwg. 0/0

Derwent Class: A41; D16; E17; F01

International Patent Class (Main): C07H-021/02; C12N-015/60

International Patent Class (Additional): C07H-021/04; C12N-001/19; C12N-001/21; C12N-009/04; C12N-009/88; C12N-015/53; C12N-015/74; C12N-015/79; C12P-007/18

9/6/1 (Item 1 from file: 155) 09265995 97457194

Glycerol conversion to 1,3-propanediol by *Clostridium pasteurianum*: cloning and expression of the gene encoding 1,3-propanediol dehydrogenase.

9/6/2 (Item 2 from file: 155) 09229632 97388589

Anaerobic pathways of glycerol dissimilation by *Enterobacter agglomerans* CNCM 1210: limitations and regulations.

9/6/3 (Item 3 from file: 155) 08016680 94377734

Phenotypic diversity of anaerobic glycerol dissimilation shown by seven *Enterobacteriaceae* species.

9/6/4 (Item 4 from file: 155) 07313946 93122543

Growth temperature-dependent activity of glycerol dehydratase in *Escherichia coli* expressing the *Citrobacter freundii* *dha* regulon.

9/6/5 (Item 5 from file: 155) 07070352 92121087

Sugar-glycerol cofermentations in *Lactobacilli*: the fate of lactate.

9/6/6 (Item 6 from file: 155) 06924196 92152855

1,3-Propanediol production by *Escherichia coli* expressing genes from the *Klebsiella pneumoniae* *dha* regulon.

9/6/7 (Item 7 from file: 155) 05901057 90155202

Anaerobic growth of *Escherichia coli* on glycerol by importing genes of the *hda* regulon from *Klebsiella pneumoniae*.

9/6/8 (Item 8 from file: 155) 05308385 87194586

*Klebsiella pneumoniae* 1,3-propanediol:NAD<sup>+</sup>-oxidoreductase.

9/6/9 (Item 9 from file: 155) 03838735 83049313

[Coenzyme properties of adenosylcobalamin analogs with modifications in the purine nucleus of the  $\alpha$ -ligand] Kofermentnye svoystva analogov adenozilkobalamina s izmenennoy purinovoyadrom  $\alpha$ '-liganda.

9/6/10 (Item 10 from file: 155) 03825037 82183110

[Substrate specificity of adenosylcobalamin-dependent glycerol dehydratase. Interaction with enantiomers of 1,2-propanediol] Substratnaya spetsifichnost' adenozilkobalamin-zavisimoy gitslerodehidratazy. Vzaimodeystvie enantiomerami 1,2-propanediola.

9/6/11 (Item 11 from file: 155) 03151140 77065853

[Effect of environmental factors on inactivation of B12-dependent glycerol dehydratase from *Aerobacter aerogenes*] Vliyaniye faktorov sredy na inaktivatsiyu B12-zavisimoy gitslerodehidratazy iz *Aerobacter aerogenes*

9/6/12 (Item 12 from file: 155) 03134432 75174520

Glycerol dehydratase from *Aerobacter aerogenes*.

9/6/13 (Item 13 from file: 155) 02620573 79062639

[Effect of the structure of the nucleoside ligand of cobalamines on their enzymatic properties in a glycerol dehydratase system] Vliyaniye struktury nukleozidnogo liganda kobalaminov na ikh kofermentnye svoystva v sisteme gitslerodehidratazy.

9/6/14 (Item 14 from file: 155) 02491634 78061052

[9-(Adenyl)alkylcobalamins as inhibitors of adenosylcobalamin-dependent glycerol dehydratase from *Aerobacter aerogenes*] 9-(Adenilil)Alkilkobalaminy kak inhibitory adenozilkobalamin-zavisimoy gitslerodehidratazy iz *Aerobacter aerogenes*.

9/6/15 (Item 15 from file: 155) 02449780 77242443

Study of the mechanism of action of adenosylcobalamin-dependent glycerol dehydratase from *Aerobacter aerogenes*. II. The inactivation kinetics of glycerol dehydratase complexes with adenosylcobalamin and its analogs.

9/6/16 (Item 16 from file: 155) 02449779 77242442

Study on the mechanism of action of adenosylcobalamin-dependent glycerol dehydratase from *Aerobacter aerogenes*. I. Role of structural components of adenosylcobalamin in the formation of the active site of glycerol dehydratase.

9/6/17 (Item 17 from file: 155) 02079984 76089220

[Role of monovalent cations in reactions catalyzed by glycerol dehydratase from *Aerobacter aerogenes*]

9/6/18 (Item 18 from file: 155) 01807381 74300091

Determination of glycerol dehydratase activity by the coupled enzymic method.

9/6/19 (Item 19 from file: 155) 01802219 74150185

[Determination of glycerol dehydratase activity by the method of coupled enzyme reactions] Opredeleniye aktivnosti gitslerodehidratazy metodom sopriazheniya fermentativnykh reaktsii.

9/6/20 (Item 20 from file: 155) 01472942 75134080

[Study of purine analogs of cobamide coenzyme in a glycerol dehydratase system from *Aerobacter aerogenes*] Izucheniye purinovykh analogov kobamidnogo kofermenta v sisteme gitslerodehidratazy iz *Aerobacter aerogenes*

9/6/21 (Item 21 from file: 155) 01424920 74269724

Allosteric interactions in glycerol dehydratase. Purification of enzyme and effects of positive and negative cooperativity for glycerol.

9/6/22 (Item 22 from file: 155) 01336562 74807057

[Formation of glycerol dehydratase by a culture of *Aerobacter aerogenes*, its partial purification and various properties] Obrazovaniye gitslerodehidratazy kulturoi *Aerobacter aerogenes*, ee chastichnaya oshchistka i nekotoryye svoystva.

9/6/23 (Item 23 from file: 155) 01244861 75002999

[Kinetics of irreversible inactivation of coenzyme and enzyme-substrate complexes of glycerol dehydratase] Kinetika neobratimoi inaktivatsii kholofermenta i ferment-substratnykh kompleksov gitslerodehidratazy

9/6/24 (Item 24 from file: 155) 01209238 73067771

[Kinetics of the transformation of 1,2-propanediol to propionic aldehyde, catalyzed by glycerol dehydratase from *Aerobacter aerogenes*] Kinetika prevrashcheniya 1,2-propanediola v propionovuyu al'degid, kataliziruemyuyu gitslerodehidratazoy iz *Aerobacter aerogenes*.

9/6/25 (Item 25 from file: 155) 01103372 70293158

Purification and properties of glycerol dehydratase.

9/6/26 (Item 26 from file: 155) 01081824 68277312

Mechanism of action of coenzyme B12-dependent glycerol dehydratase.

9/6/27 (Item 27 from file: 155) 00218268 67257076

Enzymatic determination of vitamin B12, coenzyme B12, and other cobamide derivatives in picomole quantities by means of glycerol dehydratase from *Aerobacter aerogenes*.

9/6/28 (Item 28 from file: 155) 00136925 67124546

The properties of glycerol dehydratase isolated from *Aerobacter aerogenes*, and the properties of the apoenzyme subunits.

9/6/29 (Item 1 from file: 5) 13582798 BIOSIS Number: 95582798

Biochemical and molecular characterization of coenzyme B-12-dependent glycerol dehydratase from *Citrobacter freundii* Print Number: Biological Abstracts/RRM Vol. 049ss. 007 Ref. 118404

9/6/30 (Item 2 from file: 5) 13333745 BIOSIS Number: 99333745

Physiologic mechanisms involved in accumulation of 3-hydroxypropionaldehyde during fermentation of glycerol by *Citrobacter agglomerans* Print Number: Biological Abstracts Vol. 103ss. 003 Ref. 036859

9/6/31 (Item 3 from file: 5) 12230210 BIOSIS Number: 98830210

Glycerol dehydratase activity: The limiting step for 1,3-propanediol production by *Clostridium butyricum* DSM 5431 Print Number: Biological Abstracts Vol. 101ss. 012 Ref. 180632

9/6/32 (Item 4 from file: 5) 10107492 BIOSIS Number: 95107492

FERMENTATION OF GLYCEROL TO 1,3-PROPANEDIOL IN CONTINUOUS CULTURES OF CITROBACTER-FREUNDII

9/6/33 (Item 5 from file: 5) 9107519 BIOSIS Number: 93092519

SUGAR GLYCEROL COFERMENTATIONS IN LACTOBACILLI: THE FATE OF LACTATE

9/6/34 (Item 6 from file: 5) 7479751 BIOSIS Number: 89130770

UTILIZATION OF GLYCEROL AS A HYDROGEN ACCEPTOR BY LACTOBACILLUS-REUTERI: PURIFICATION OF 1,3-PROPANEDIOL NAD OXIDOREDUCTASE

9/6/35 (Item 7 from file: 5) 7479748 BIOSIS Number: 89130767

PURIFICATION AND CHARACTERIZATION OF GLYCEROL DEHYDRATASE FROM LACTOBACILLUS-REUTERI

9/6/36 (Item 8 from file: 5) 4521051 BIOSIS Number: 78094874

ANAEROBIC REDUCTION OF GLYCEROL TO 1,3-PROPANEDIOL BY LACTOBACILLUS-BREVIS AND LACTOBACILLUS-BUCHNERI

9/6/37 (Item 9 from file: 5) 4402667 BIOSIS Number: 77077994

COBALTCORRINOIDS: THE DERIVATIVES OF VITAMIN B-12 PSEUDOFORMS AS CORRINOID ENZYME INHIBITORS

9/6/38 (Item 10 from file: 5) 4347088 BIOSIS Number: 77022415

SOME PHYSICO-CHEMICAL FEATURES GLYCEROL DEHYDRATASE CATALYZED REACTIONS

9/6/39 (Item 11 from file: 5) 4343221 BIOSIS Number: 77018548

PRODUCTION OF 3-HYDROXY-PROPIONALDEHYDE FROM GLYCEROL

9/6/40 (Item 12 from file: 5) 4167203 BIOSIS Number: 26019546

COENZYME PROPERTIES OF ADENOSYL COBALAMIN ANALOGS WITH A CHANGED PURINE NUCLEUS OF THE ALPHA-LIGAND

9/6/41 (Item 13 from file: 5) 4079098 BIOSIS Number: 76028949

COENZYME PROPERTIES OF ADENOSYL COBALAMIN ANALOGS WITH MODIFICATIONS IN THE ALPHA-LIGAND

9/6/42 (Item 14 from file: 5) 3847492 BIOSIS Number: 24054851

SUBSTRATE SPECIFICITY OF ADENOSYL COBALAMIN-DEPENDENT GLYCEROL DEHYDRATASE EC-4.2.1.30: INTERACTION WITH ENANTIOMERS OF 1,2-PROPANEDIOL

9/6/43 (Item 15 from file: 5) 3693569 BIOSIS Number: 73085936

GLYCEROL FERMENTATION IN *KLEBSIELLA-PNEUMONIAE*: FUNCTIONS OF THE COENZYME-B12-DEPENDENT GLYCEROL AND DIOL DEHYDRATASES

9/6/44 (Item 16 from file: 5) 2974635 BIOSIS Number: 69012042  
INTERACTION OF SUBSTRATES AND THEIR ANALOGS WITH ADENOSYL COBALAMIN DEPENDENT GLYCEROL DEHYDRATASE

9/6/45 (Item 17 from file: 5) 2944727 BIOSIS Number: 19049636  
PARTICIPATION OF CYCLIC AMP IN REGULATION OF COENZYME B-12 DEPENDENT GLYCEROL DEHYDRATASE EC-4.2.1.30 SYNTHESIS FROM KLEBSIELLA-PNEUMONIAE ATCC-25955

9/6/46 (Item 18 from file: 5) 2944720 BIOSIS Number: 19049629  
ADENOSYL COBALAMIN DEPENDENT GLYCEROL DEHYDRATASE EC-4.2.1.30 INTERACTION WITH SUBSTRATES AND THEIR ANALOGS

9/6/47 (Item 19 from file: 5) 2937208 BIOSIS Number: 19042117  
ENZYMATIC ESTIMATION OF VITAMIN B-12

9/6/48 (Item 20 from file: 5) 2856392 BIOSIS Number: 18028803  
INTERACTION OF SUBSTRATES AND THEIR ANALOGS WITH ADENOSYL COBALAMIN DEPENDENT GLYCEROL DEHYDRATASE EC-4.2.1.30

9/6/49 (Item 21 from file: 5) 2835422 BIOSIS Number: 18007833  
EFFECT OF STRUCTURE OF NUCLEOSIDE LIGAND OF COBALAMINS ON THEIR COENZYME PROPERTIES IN THE GLYCEROL DEHYDRATASE EC-4.2.1.30 SYSTEM

9/6/50 (Item 22 from file: 5) 2782252 BIOSIS Number: 68037159  
EFFECT OF THE NUCLEOSIDE LIGAND STRUCTURE OF COBALAMINS ON THEIR COENZYMIC PROPERTIES IN THE GLYCEROL DEHYDRATASE SYSTEM

9/6/51 (Item 23 from file: 5) 2775647 BIOSIS Number: 68030554  
SEARCH FOR NEW MEDICINAL PREPARATIONS ON THE BASIS OF VITAMIN B-12 DERIVATIVES SYNTHESIS AND STUDY OF THE PHYSICO-CHEMICAL AND COENZYME PROPERTIES OF ADENOSYL COBALAMIN DERIVATIVES

9/6/52 (Item 24 from file: 5) 2106317 BIOSIS Number: 63010737  
THE ROLE OF THE PROPANAMIDE GROUP OR THE CORRIN MACRO CYCLE IN THE MANIFESTATION OF COENZYMIC PROPERTIES OF THE COBAMIDE COENZYME

9/6/53 (Item 25 from file: 5) 1666115 BIOSIS Number: 60010683  
STUDY OF PURINE ANALOGS OF THE COBAMIDE COENZYME IN THE GLYCEROL DEHYDRATASE SYSTEM FROM AEROBACTER-AEROGENES

9/6/54 (Item 1 from file: 73) 8406923 EMBASE No: 92083103  
Sugar-glycerol cofermentations in lactobacilli: The fate of lactate

9/6/55 (Item 2 from file: 73) 8412604 EMBASE No: 87149266  
Klebsiella pneumoniae 1,3-propanediol:NAD<sup>+</sup> oxidoreductase

9/6/56 (Item 3 from file: 73) 1264966 EMBASE No: 79032619  
Effects of the nucleoside ligands structure of cobalamines on their coenzymic properties in glycerol dehydratase

9/6/57 (Item 4 from file: 73) 1000051 EMBASE No: 78170429  
Study on the mechanism of action of adenosylcobalamin-dependent glycerol dehydratase from Aerobacter aerogenes. II. The inactivation kinetics of glycerol dehydratase complexes with adenosylcobalamin and its analogs

9/6/58 (Item 5 from file: 73) 1000050 EMBASE No: 78170428  
Study on the mechanism of action of adenosylcobalamin-dependent glycerol dehydratase from Aerobacter aerogenes. I. Role of structural components of adenosylcobalamin in the formation of the active site of glycerol dehydratase

9/6/59 (Item 6 from file: 73) 859479 EMBASE No: 78025357  
Influence of environmental factors on the inactivation of B<sub>12</sub> dependent glycerol dehydratase from Aerobacter aerogenes

9/6/60 (Item 7 from file: 73) 630679 EMBASE No: 77007407  
The role of monovalent cations in reactions catalyzed by glycerol dehydratase from Aerobacter aerogenes

9/6/61 (Item 8 from file: 73) 516161 EMBASE No: 93310393  
Response to vasoactive neuropeptides in basilar arteries isolated from stroke-prone spontaneously hypertensive rats

9/6/62 (Item 9 from file: 73) 466469 EMBASE No: 76048032  
The interaction of apoglycerol dehydratase from Aerobacter aerogenes with 'apurine' analogs of cobamide coenzyme

9/6/63 (Item 10 from file: 73) 444734 EMBASE No: 76025321  
Production of glycerol dehydratase by culture of Aerobacter aerogenes, its partial purification, and some properties

9/6/64 (Item 11 from file: 73) 372001 EMBASE No: 75167006  
Investigation of purine analogues of the cobamide coenzyme in the glycerol dehydratase system from Aerobacter aerogenes (Russian)

9/7/1 (Item 1 from file: 155) DIALOG(R) File 155: MEDLINE(R) (c) format only 1997 Knight-Ridder Info. All rights reserved.  
09265995 97457194

Glycerol conversion to 1,3-propanediol by *Clostridium pasteurianum*: cloning and expression of the gene encoding 1,3-propanediol dehydrogenase.

Luers F; Seyfried M; Daniel R; Gottschalk G

Institut für Mikrobiologie der Georg-August-Universität, Göttingen, Germany.

FEMS Microbiol Lett (NETHERLANDS) Sep 15 1997, 154 (2) p337-45, ISSN 0378-1097 Journal Code: FMLS Languages: ENGLISH Document type: JOURNAL ARTICLE

When grown on glycerol as sole carbon and energy source, cell extracts of *Clostridium pasteurianum* exhibited activities of glycerol dehydrogenase, dihydroxyacetone kinase, glycerol dehydratase and 1,3-propanediol dehydrogenase. The genes encoding the latter two enzymes were cloned by colony hybridization using the *dhaT* gene of *Citrobacter freundii* as a heterologous DNA probe and expressed in *Escherichia coli*. The native molecular mass of 1,3-propanediol dehydrogenase (DhaT) is 440,000 Da. The *dhaT* gene of *C. pasteurianum* was subcloned and its nucleotide sequence (1158 bp) was determined. The deduced gene product (41,776 Da) revealed high similarity to DhaT of *C. freundii* (80.5% identity; 89.8% similarity).

9/7/4 (Item 4 from file: 155) DIALOG(R) File 155: MEDLINE(R) (c) format only 1997 Knight-Ridder Info. All rights reserved.

07313946 93122543

Growth temperature-dependent activity of glycerol dehydratase in *Escherichia coli* expressing the *Citrobacter freundii* *dha* regulon.

Daniel R; Gottschalk G

Institute für Mikrobiologie, Georg-August-Universität, Göttingen, FRG.

FEMS Microbiol Lett (NETHERLANDS) Dec 15 1992, 79 (1-3) p281-5, ISSN 0378-1097 Journal Code: FMLS Languages: ENGLISH Document type: JOURNAL ARTICLE

Using the cosmid pWE15, a genomic library of *Citrobacter freundii* DNA in *Escherichia coli* ECL707 was prepared and screened for glycerol utilization. Six out of approximately 3000 clones were positive. One clone, harboring the recombinant cosmid pRD1, expressed glycerol dehydratase in high activity when grown at 28 degrees C but not at 37 degrees C. The growth temperature had little effect on the activity of the other enzymes encoded by the *dha* regulon. When the glycerol-containing medium was supplemented with corrinoids, the recombinant *E. coli* strain produced 1,3-propanediol in high amounts at 28 degrees C.

9/7/5 (Item 5 from file: 155) DIALOG(R) File 155: MEDLINE(R) (c) format only 1997 Knight-Ridder Info. All rights reserved.

07070352 92121087

Sugar-glycerol cofermentations in lactobacilli: the fate of lactate.

Veiga da Cunha M; Foster MA

Department of Biochemistry, University of Oxford, United Kingdom.

J Bacteriol (UNITED STATES) Feb 1992, 174 (3) p1013-9, ISSN 0021-9193 Journal Code: HH3 Languages: ENGLISH Document type: JOURNAL ARTICLE

The simultaneous fermentation of glycerol and sugar by *Lactobacillus brevis* B22 and *Lactobacillus buchneri* B190 increases both the growth rate and total growth. The reduction of glycerol to 1,3-propanediol by the lactobacilli was found to influence the metabolism of the sugar cofermented by channeling some of the intermediate metabolites (e.g., pyruvate) towards NADH-producing (rather than NADH-consuming) reactions. Ultimately, the absolute requirement for NADH to prevent the accumulation of 3-hydroxypropionaldehyde leads to a novel lactate-glycerol cofermentation. As a result, additional ATP can be made not only by (i) converting pyruvate to acetate via acetyl phosphate rather than to the ethanol usually found and (ii) oxidizing part of the intermediate pyruvate to acetate instead of the usual reduction to lactate but also by (iii) reoxidation of accumulated lactate to acetate via pyruvate. The conversion of lactate to pyruvate is probably catalyzed by NAD-independent lactate dehydrogenases that are found only in the cultures oxidizing lactate and producing 1,3-propanediol, suggesting a correlation between the expression of these enzymes and a raised intracellular NAD/NADH ratio. The enzymes metabolizing glycerol (glycerol dehydratase and 1,3-propanediol dehydrogenase) were expressed in concert without necessary induction by added glycerol, although their expression may also be influenced by the intracellular NAD/NADH ratio set by the different carbohydrates fermented.

9/7/8 (Item 8 from file: 155) DIALOG(R) File 155: MEDLINE(R) (c) format only 1997 Knight-Ridder Info. All rights reserved.

05308385 87194586

Klebsiella pneumoniae 1,3-propanediol:NAD<sup>+</sup> oxidoreductase.

Johnson EA; Lin EC

J Bacteriol (UNITED STATES) May 1987, 169 (5) p2050-4, ISSN 0021-9193 Journal Code: HH3 Contract/Grant No.: 5-R01-GM11983, GM, NIGMS Languages: ENGLISH Document type: JOURNAL ARTICLE  
 Fermentative utilization of glycerol, a more reduced carbohydrate than sugars and ketoses, requires the disposal of the two extra hydrogen atoms. This is accomplished by sacrificing an equal quantity of glycerol via an auxiliary pathway initiated by glycerol dehydratase. The product, 3-hydroxypropionaldehyde, is then reduced by 1,3-propanediol NAD<sup>+</sup>:oxidoreductase (1,3-propanediol dehydrogenase; EC 1.1.1.202), resulting in the regeneration of NAD<sup>+</sup> from NADH. The pathway for the assimilation of glycerol is initiated by an NAD-linked dehydrogenase. In *Klebsiella pneumoniae* the two pathways are encoded by the *dha* regulon which is inducible only anaerobically. In this study 1,3-propanediol:NAD<sup>+</sup>:oxidoreductase was purified from cells grown anaerobically on glycerol. The enzyme was immunochemically distinct from the NAD-linked glycerol dehydrogenase and was an octamer or hexamer of a polypeptide of 45,000 +/- 3,000 daltons. When tested as a dehydrogenase, only 1,3-propanediol served as a substrate; no activity was detected with ethanol, 1-propanol, 1,2-propanediol, glycerol, or 1,4-butanediol. The enzyme was inhibited by chelators of divalent cations. An enzyme preparation inhibited by alpha,alpha'-dipyridyl was reactivated by the addition of Fe<sup>2+</sup> or Mn<sup>2+</sup> after removal of the chelator by gel filtration. As for glycerol dehydrogenase, 1,3-propanediol oxidoreductase is apparently inactivated by oxidation during aerobic metabolism, under which condition the enzyme becomes superfluous.

9/7/12 (Item 12 from file: 155) IALOG(R)File 155:MEDLINE(R) (c) format only 1997 Knight-Ridder Info. Allrts. reserv.  
 03134432 75174520

Glycerol dehydratase from *Aerobacter aerogenes*.

Johnson BC; Stroinski A; Schneider Z

Methods Enzymol (UNITED STATES) 1975, 42 p315-23, ISSN 0076-6879 Journal Code: MVA Languages: ENGLISH Document type: JOURNAL ARTICLE

9/7/29 (Item 1 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

13582798 BIOSIS Number: 99582798

Biochemical and molecular characterization of coenzyme B-12-dependent glycerol dehydratase from *Citrobacter freundii*

Daniel R; Seyfried M; Gottschalk G

Inst. Mikrobiol. Georg-August-Univ. Goettingen, Grisebachstr. 8, 37077 Goettingen, Germany

Abstracts of the General Meeting of the American Society for Microbiology 97 (0), 1997. 353. Full Journal Title: 97th General Meeting of the American Society for Microbiology, Miami Beach, Florida, USA, May 4-8, 1997.

Abstracts of the General Meeting of the American Society for Microbiology ISSN: 1060-2011 Language: ENGLISH Print Number: Biological Abstracts/RRM Vol. 049 Iss. 007 Ref. 118404

9/7/31 (Item 3 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

12230210 BIOSIS Number: 98830210

Glycerol dehydratase activity: The limiting step for 1,3-propanediol production by *Clostridium butyricum* DSM 5431

Abbad-Andaloussi S; Guedon E; Spiesser E; Petitdemange H

Lab. Chimie Biol. I, Univ. Henri Poincaré Nancy I, BP 239, 54506 Vandœuvre-les-Nancy Cedex, France

Letters in Applied Microbiology 22 (4), 1996. 311-314. Full Journal Title: Letters in Applied Microbiology ISSN: 0266-8254 Language: ENGLISH Print Number: Biological Abstracts Vol. 101 Iss. 012 Ref. 180632

Glycerol catabolism by *Clostridium butyricum* DSM 5431 into acetate, butyrate and 1,3-propanediol (1,3-PD) was studied in chemostat culture. The fact that the intracellular concentrations of NADH (18-22 µmol g<sup>-1</sup> dry cell mass) were extremely high suggested that the dehydratase activity was the rate limiting step in 1,3-PD formation. This limitation was proved by the addition of propionaldehyde, another substrate of propanediol dehydrogenase, into the culture medium. This resulted in an increase in (i) glycerol utilization, (ii) biomass formation and (iii) product biosynthesis.

9/7/32 (Item 4 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

10107492 BIOSIS Number: 95107492

FERMENTATION OF GLYCEROL TO 1,3-PROPANEDIOL IN CONTINUOUS CULTURES OF CITROBACTER-FREUNDII

BOENIGK R; BOWIEN S; GOTTSCHALK G

INSTITUT FUER MIKROBIOLOGIE, GEORG-AUGUST-UNIVERSITAET GOETTINGEN, GRISEBACHSTRASSE 8, W-3400 GOETTINGEN, GERMANY.

APPL MICROBIOL BIOTECHNOL 38 (4), 1993. 453-457. CODEN: AMBID Full Journal Title: Applied Microbiology and Biotechnology Language: ENGLISH

The conversion of glycerol to 1,3-propanediol by *Citrobacter freundii* DSM 30040 was optimized in single- or two-stage continuous cultures. The productivity of 1,3-propanediol formation was higher under glycerol limitation and increased with the dilution rate (D) to a maximum of 3.7 g. cndtot. l<sup>-1</sup> .cndtot. h<sup>-1</sup>. Glycerol dehydratase seemed to be the rate-limiting step in 1, 3-propane-diol formation. Conditions for the two-stage fermentation process were as follows: first stage, glycerol limitation (250 mM), pH 7.2, D = 0.1 h<sup>-1</sup>, 32. degree. C; second stage, additional glycerol, pH 6.6, D = 0.05 h<sup>-1</sup>, 28. degree. C. Under these conditions 876 mM glycerol were consumed, the final 1,3-propanediol concentrations was 545 mM, and the overall productivity. 1.38 g. cndtot. l<sup>-1</sup> .cndtot. h<sup>-1</sup>.

9/7/33 (Item 5 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

9107519 BIOSIS Number: 93092519

SUGAR GLYCEROL COFERMENTATIONS IN LACTOBACILLI THE FATE OF LACTATE

VEIGA DA CUNHA M; FOSTER M A

MICROBIOL. UNIT, DEP. BIOCHEM., UNIV. OXFORD, OXFORD OX1 3QU, UK.

J BACTERIOL 174 (3), 1992. 1013-1019. CODEN: JOBAA Full Journal Title: Journal of Bacteriology Language: ENGLISH

The simultaneous fermentation of glycerol and sugar by *Lactobacillus brevis* B22 and *Lactobacillus buchneri* B190 increases both the growth rate and total growth. The reduction of glycerol to 1,3-propanediol by the lactobacilli was found to influence the metabolism of the sugarcofermented by channelling some of the intermediate metabolites (e.g., pyruvate) towards NADH-producing (rather than NADH-consuming) reactions. Ultimately, the absolute requirement for NADH to prevent the accumulation of 3-hydroxypropionaldehyde leads to a novel lactate-glycerolcofermentation. As a result, additional ATP can be made not only by (i) converting pyruvate to acetate via acetyl phosphate rather than to the ethanol usually found and (ii) oxidizing part of the intermediate pyruvate to acetate instead of the usual reduction to lactate but also by (iii) reoxidation of accumulated lactate to acetate via pyruvate. The conversion of lactate to pyruvate is probably catalyzed by NAD-independent lactate dehydrogenases that are found only in the cultures oxidizing lactate and producing 1,3-propanediol, suggesting a correlation between the expression of these enzymes and a raised intracellular NAD/NADH ratio. The enzymes metabolizing glycerol (glycerol dehydratase and 1,3-propanediol dehydrogenase) were expressed in concert without necessary induction by added glycerol, although their expression may also be influenced by the intracellular NAD/NADH ratio set by the different carbohydrates fermented.

9/7/35 (Item 7 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

7479748 BIOSIS Number: 89130767

PURIFICATION AND CHARACTERIZATION OF GLYCEROL DEHYDRATASE FROM LACTOBACILLUS-REUTERI

TALARICO T L; DOBROGOSZ W J

DEP. MICROBIOL., NORTH CAROLINA STATE UNIV., RALEIGH, N.C. 27695.

APPL ENVIRON MICROBIOL 56 (4), 1990. 1195-1197. CODEN: AEMID Full Journal Title: Applied and Environmental Microbiology Language: ENGLISH

A coenzyme B12-dependent glycerol dehydratase from *Lactobacillus reuteri* has been purified and characterized. The dehydratase has a molecular weight of approximately 200,000, and sodium dodecyl sulfate-polycrylamide gel electrophoresis yielded a single major band with a molecular weight of 52,000. Km values for substrates and coenzyme B12 were in the millimolar and the submicromolar range, respectively.

9/7/36 (Item 8 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

4521051 BIOSIS Number: 78094874

ANAEROBIC REDUCTION OF GLYCEROL TO 1,3-PROPANEDIOL BY LACTOBACILLUS-BREVIS AND LACTOBACILLUS-BUCHNERI

SCHUETZ H; RADLER F

INSTITUT FUER MIKROBIOLOGIE UND WEINFORSCHUNG, UNIVERSITAET MAINZ, POSTFACH 3980, D-6500 MAINZ.

SYST APPL MICROBIOL 5 (2), 1984. 169-178. CODEN: SAMID Full Journal Title: Systematic and Applied Microbiology Language: ENGLISH

Three strains of *L. brevis* and 1 strain of *L. buchneri* grew very poorly on glucose. Good growth was observed on glucose plus glycerol; while glucose was fermented to acetate or ethanol, lactate and CO<sub>2</sub>, glycerol was dehydrated to 3-hydroxypropanal and subsequently reduced to 1,3-propanediol. Cell extracts of *L. brevis* and *L. buchneri* grown on glucose plus glycerol contained a B12-dependent glycerol dehydratase and a 1,3-propanediol dehydrogenase. Glycerol was not metabolized when used as the only substrate. Fructose as sole C source was partially reduced to mannitol. The joint fermentation of fructose and glycerol yielded 1,3-propanediol from glycerol. Ribose was fermented but did not support glycerol fermentation. Extracts from ribose grown cells did not contain glycerol dehydratase or 1,3-propanediol dehydrogenase. Besides glycerol the following diols were metabolized as cosubstrates with glucose: 1,2-propanediol, ethylene glycol and 2,3-butanediol yielding 1-propanol, ethanol and 2-butanol, respectively. Washed cells of *L. brevis* strains B 18 and B 20 formed 1,3-propanediol and 1,2-propanediol from glycerol; the third strain, B 22, formed only 1,2-propanediol from glycerol in the absence of glucose.

9/7/38 (Item 10 from file: 5) DIALOG(R)File 5:BIOSIS PREVIEWS(R) (c) 1997 BIOSIS. Allrts. reserv.

4347088 BIOSIS Number: 77022415

SOME PHYSICO-CHEMICAL FEATURES GLYCEROL DEHYDRATASE CATALYZED REACTIONS

POZNANSKAYA A A; KOROSOVA T L

SCI.-PROD. ASSOC. "VITAM.", MOSCOW, USSR.

BIOKHIMIYA 48 (4), 1983. 539-543. CODEN: BIOHA Full Journal Title: Biokhimiya Language: RUSSIAN

The concentration of active centers in preparations of B12-dependent glycerol dehydratase from *Klebsiella pneumoniae* was determined by their titration with the coenzyme, adenosylcobalamine (AdoCbl). Some kinetic and thermodynamic features of the reactions catalyzed by the enzyme were established. The data obtained are indicative of a significant contribution of hydrophobic interactions to the substrate and AdoCbl binding to glycerol dehydratase.

9/7/54 (Item 1 from file: 73) DIALOG(R)File 73:EMBASE (c) 1997 Elsevier Science B.V. Allrts. reserv.

8406923 EMBASE No: 92083103

Sugar-glycerol cofermentations in lactobacilli: The fate of lactate

Da Cunha M.V.; Foster M.A.

Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU United Kingdom

J. BACTERIOL. (USA), 1992, 174/3 (1013-1019) CODEN: JOBAA ISSN: 0021-9193 LANGUAGES: English SUMMARY LANGUAGES: English

The simultaneous fermentation of glycerol and sugar by *Lactobacillus brevis* B22 and *Lactobacillus buchneri* B190 increases both the growth rate and total growth. The reduction of glycerol to 1,3-propanediol by the lactobacilli was found to influence the metabolism of the sugar cofermented by channelling some of the intermediate metabolites (e.g., pyruvate) towards NADH-producing (rather than NADH-consuming) reactions. Ultimately, the absolute requirement for NADH to prevent the accumulation of 3-hydroxypropionaldehyde leads to a novel lactate-glycerobolfermentation. As a result, additional ATP can be made not only by (i) converting pyruvate to acetate via acetyl phosphate rather than to the ethanol usually found and (ii) oxidizing part of the intermediate pyruvate to acetate instead of the usual reduction to lactate but also by (iii) reoxidation of accumulated lactate to acetate via pyruvate. The conversion of lactate to pyruvate is probably catalyzed by NAD-independent lactate dehydrogenases that are found only in the cultures oxidizing lactate and producing 1,3-propanediol, suggesting a correlation between the expression of these enzymes and a raised intracellular NAD/NADH ratio. The enzymes metabolizing glycerol (glycerol dehydratase and 1,3-propanediol dehydrogenase) were expressed in concert without necessary induction by added glycerol, although their expression may also be influenced by the intracellular NAD/NADH ratio set by the different carbohydrates fermented.

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(FILE 'USPAT' ENTERED AT 13:10:59 ON 09 DEC 1997)

L1 13 S (DIOL OR GLYCEROL) (2N)(DEHYDRASE OR DEHYDRATASE)

1. 5,686,276, Nov. 11, 1997, Bioconversion of a fermentable carbon source to 1,3-propanediol by a single microorganism; Lisa Anne Laffend, et al., 435/158, 252.31, 252.33 :IMAGE AVAILABLE:
2. 5,633,362, May 27, 1997, Production of 1,3-propanediol from glycerol by recombinant bacteria expressing recombinant "diol" "dehydratase"; Vasantha Nagarajan, et al., 536/23.1, 435/252.3, 252.33; 536/22.1, 24.3 :IMAGE AVAILABLE:
3. 5,599,689, Feb. 4, 1997, Process for making 1,3-propanediol from carbohydrates using mixed microbial cultures; Sharon L. Haynie, et al., 435/42, 158 :IMAGE AVAILABLE:
4. 5,589,372, Dec. 31, 1996, Squalene synthetase; Gordon W. Robinson, 435/193, 252.3, 254.11, 320.1, 348, 355, 358, 365; 536/23.2, 24.3 :IMAGE AVAILABLE:
5. 5,480,641, Jan. 2, 1996, Feed additive which consists of whey and Lactobacillus reuteri and a method of delivering Lactobacillus reuteri to the gastrointestinal tract; Ivan A. Casas-Perez, 424/93.45, 93.4; 426/61; 435/252.9, 853 :IMAGE AVAILABLE:
6. 5,458,875, Oct. 17, 1995, In ovo method for delivering Lactobacillus reuteri to the gastrointestinal tract of poultry; Ivan A. Casas-Perez, et al., 424/93.45; 119/6.8; 424/93.4; 435/252.1, 252.9 :IMAGE AVAILABLE:
7. 5,439,678, Aug. 8, 1995, Method for inhibiting microorganism growth; Walter J. Dobrogosz, et al., 424/93.45, 93.4; 426/61; 435/34, 123, 244, 252.1; 514/693 :IMAGE AVAILABLE:
8. 5,413,960, May 9, 1995, Antibiotic reuterin; Walter J. Dobrogosz, et al., 435/189, 124, 184 :IMAGE AVAILABLE:
9. 5,405,839, Apr. 11, 1995, Vitamin B.sub.12 derivative, preparation process thereof, and use thereof; Tetsuo Toraya, et al., 514/52; 536/26.4, 26.41 :IMAGE AVAILABLE:
10. 5,352,586, Oct. 4, 1994, Method of determining the presence of an antibiotic produced by Lactobacillus reuteri; Walter J. Dobrogosz, et al., 435/34, 41, 124, 183, 252.1, 853 :IMAGE AVAILABLE:
11. 5,164,309, Nov. 17, 1992, Process for the microbiological preparation of 1,3-propane-diol from glycerol by citrobacter; G. Gottschalk, et al., 435/158, 252.1 :IMAGE AVAILABLE:
12. 4,962,027, Oct. 9, 1990, Production of 3-hydroxypropionaldehyde from glycerol by Klebsiella pneumoniae; Patricia J. Silinger, et al., 435/147, 155, 244, 252.1 :IMAGE AVAILABLE:
13. 4,235,869, Nov. 25, 1980, Assay employing a labeled Fab-fragment ligand complex; Moshe Schwarzbarg, 436/512; 250/302; 435/7.7, 7.72, 968; 436/513, 536, 537, 541, 800 :IMAGE AVAILABLE:

US PAT NO: 5,633,362 :IMAGE AVAILABLE: L1: 2 of 13

#### ABSTRACT:

A process is provided for the bioconversion of glycerol to 1,3-propanediol in which genes from a bacteria known to possess "diol" "dehydratase" enzyme for 1,2-propanediol degradation are cloned into a bacterial host and the host is grown in the presence of glycerol; expression of the foreign genes in the host cell facilitates the enzymatic conversion of glycerol to 1,3-propanediol which is isolated from the culture.

#### What is claimed is:

1. A cosmid comprising a DNA fragment of about 35 kb isolated from Klebsiella pneumoniae wherein said fragment encodes an active "diol" "dehydratase" enzyme having the restriction digest in FIG. 5, columns numbered 4, said cosmid contained within a transformed E. coli deposited with the American Type Culture Collection under accession number ATCC 69790.
2. A transformed microorganism comprising a host microorganism and the cosmid of claim 1.
3. The transformed microorganism of claim 2 wherein the host microorganism is E. coli, and which is deposited with the American Type Culture Collection as accession number ATCC 69790.
4. The cosmid of claim 1 which when transformed into bacteria causes metabolism of glycerol to 1,3-propanediol.
5. A transformed microorganism comprising a host microorganism and a DNA fragment of the cosmid of claim 1, said fragment encoding an active functional protein.
6. A DNA fragment comprising a gene encoding a "diol" "dehydratase" enzyme, said gene encompassed by the cosmid of claim 1.
7. A isolated gene encoding an active "diol" "dehydratase" enzyme comprising a contiguous sequence which consists of SEQ ID NO: 1.
8. A isolated gene encoding an active alcohol dehydrogenase comprising a contiguous sequence which consists of SEQ ID NO: 2.
9. A transformed microorganism comprising a host microorganism and the heterologous gene of claim 7 or claim 8.
10. A transformed microorganism comprising E. coli DH5.alpha. and the DNA sequence of claim 7 or claim 8.

US PAT NO: 5,164,309 :IMAGE AVAILABLE: L1: 11 of 13

#### ABSTRACT:

A process of the microbiological preparation of 1,3 propanediol from glycerol in growth media of suitable bacterial strains is described, accompanied by the addition of a substrate in the form of a H-donor and the separation of the propanediol formed. It is characterized in that a) biomass is formed in a growth phase from the selected bacterial strain and accompanied by feeding with glycerol and, if necessary, while substantially excluding the H-donor until a stationary growth phase occurs and b) further glycerol and H-donor matched to the biomass are added to the resulting stationary cell suspension for increased 1,3-propanediol formation. This process makes it possible to produce 1,3-propanediol in a high yield from glycerol with a small amount of unobjectionable by-products in batchwise manner or in continuous form, following immobilization.

#### We claim:

1. In a process for the microbiological preparation of 1,3-propanediol by cultivating in a growth medium containing glycerol and a bacterial strain which is able to convert the glycerol into 1,3-propanediol and isolating the 1,3-propanediol thus obtained, the improvement which comprises the steps of:
  - (i) forming a biomass by culturing a bacterial strain from the Citrobacter genus in the growth medium containing glycerol, wherein the formation of the biomass is carried out with the substantial exclusion of any H donor; permitting the bacterial cells to reach a stationary cell phase; thereafter adding to said biomass additional glycerol and a sugar as an H-donor to the biomass, while keeping the cells in essentially a stationary phase; and
  - (iv) then isolating the 1,3-propanediol thus prepared.
2. The process according to claim 1 wherein said strain is a strain of Citrobacter freundii.
3. The process according to claim 1 wherein step (i) is performed under anaerobic conditions.
4. The process according to claim 1 wherein step (ii) is performed under anaerobic conditions.
5. The process according to claim 1 wherein a pH-value of approximately 6.5 to 8.5 is maintained in steps (i) and (iii).
6. The process according to claim 1 wherein steps (i) and (iii) are performed in a mineral medium.
7. The process according to claim 1 wherein step (i) is concluded by the addition of a predetermined quantity of phosphate or nitrogen source.

8. The process according to claim 7 wherein an ammonium salt is used as the nitrogen source or a potassium dihydrogen phosphate is used as the phosphate source.

9. The process according to claim 1 wherein glycerol is initially present in step (ii) in the amount of 0.2 to 1.5 molar concentration.

10. The process according to claim 1 wherein glycerol is initially present in step (i) in approximately 0.1 to 0.4 molar concentration.

11. The process according to claim 1 wherein said biomass obtained in step (i) is immobilized before step (iii).

12. The process according to claim 11 wherein said immobilization is carried out with calcium alginate.

US PAT NO: 4,962,027 :IMAGE AVAILABLE: L1: 12 of 13

#### ABSTRACT:

A method is disclosed for producing 3-hydroxypropionaldehyde (3-HPA) from glycerol by culturing the bacterium *Klebsiella pneumoniae* having the identifying characteristics of NRRL B-4011, under aerobic conditions, in an aqueous nutrient medium containing glycerol and a compound that causes 3-HPA to be accumulated by blocking the conversion of 3-HPA to trimethylene glycol. This process is particularly useful for the production, from renewable resources, of acrylic acid, an industrially important polymerizable monomer used in the manufacture of synthetic polymers and plastics and which is presently derived from fossil fuel sources.

#### We claim:

1. A method for the production of 3-hydroxypropionaldehyde (3-HPA) from glycerol, which comprises culturing the bacterium *Klebsiella pneumoniae* NRRL B-4011 or subcultures thereof, under aerobic conditions, in an aqueous nutrient medium containing an amount of glycerol effective for the induction of "glycerol" dehydratase and the production of a recoverable quantity of 3-HPA, and an amount of semicarbazide hydrochloride sufficient to prevent the conversion of 3-HPA to trimethylene glycol, until a recoverable quantity of 3-HPA is produced.

2. The method of claim 1 wherein said bacterium is first grown in an aqueous nutrient medium containing a carbon source which induces the production of dehydratase enzyme and further incubated in an aqueous medium containing glycerol and semicarbazide hydrochloride.

3. The method of claim 2 wherein said carbon source is glycerol, 1,2-propanediol, or 1,2-ethanediol.